84745

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 182 207 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
27.02.2002 Bulletin 2002/09

(51) Int Cl.[7]: **C07K 5/062**, C07K 5/078, A61K 38/05

(21) Application number: 00126874.7

(22) Date of filing: 07.12.2000

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: 11.08.2000 US 637483

(71) Applicant: CORVAS INTERNATIONAL, INC.
San Diego, CA 92121-1102 (US)

(72) Inventors:
- Semple, Joseph Edward
  San Diego, CA 92131 (US)
- Weinhouse, Michael I.
  Escondido, CA 92029 (US)
- Levy, Odile Ester
  San Diego, CA 92130 (US)
- Madison, Edwin L.
  San Diego, CA 92121 (US)

(74) Representative: VOSSIUS & PARTNER
Siebertstrasse 4
81675 München (DE)

(54) Non-covalent inhibitors of urokinase and blood vessel formation

(57) Novel compounds having activity as non-covalent inhibitors of urokinase and having activity in reducing or inhibiting blood vessel formation are provided. These compounds have P1 a group having an amidino or guanidino moiety or derivative thereof. These compounds are useful in vitro for monitoring plasminogen activator levels and *in vivo* in treatment of conditions which are ameliorated by inhibition of or decreased activity of urokinase and in treating pathologic conditions wherein blood vessel formation is related to a pathologic condition.

Printed by Jouve, 75001 PARIS (FR)

Description

[0001] Urokinase is an enzyme involved in the metastasis of tumor cells, neovascularization, and other activities. One purpose of the present invention is to provide novel compounds which are active as inhibitors of urokinase that can be used to inhibit the activity of urokinase and thereby attenuate its deleterious effects. Another purpose of the present invention is to provide novel compounds which inhibit blood vessel formation, particularly blood vessel formation related to a pathologic condition.

[0002] Urinary-type plasminogen activator (uPA; urokinase) is a serine protease within the trypsin/chymotrypsin family. In its physiological state, uPA is found in three forms: single chain pro-uPA, two chain uPA, and low molecular weight uPA (lacks N-terminal domains). The zymogen, pro-uPA, is converted to u-PA by cleavage of the peptide bond at K158-I159. The resultant two chain uPA is linked by disulfide bridges, has an M_r of about 50 kD, and a C-terminal serine proteinase domain.

[0003] The activity of uPA is focused to cell surfaces upon binding to its receptor, uPAR. uPAR is a single-chain glycosyl phosphatidyl inositol (GPI)-anchored membrane receptor. The N-terminal 92 amino acids of uPAR play a dominant role in binding to uPA and pro-uPA. Receptor for uPA has been located on T-cells, NK cells, monocytes, and neutrophils, as well as vascular endothelial cells, fibroblasts, smooth muscle cells, keratinocytes, placental trophoblasts, hepatocytes, and a wide variety of tumor cells.

[0004] After conversion of pro-uPA to uPA, which occurs primarily at the uPAR on the cell surface, uPA activates plasminogen to plasmin. Activation occurs upon cleavage at residues PGR-W for human plasminogen, or at residues SGR-IV for bovine plasminogen. Because plasminogen also is present on the cell surface, this activation cascade focuses the activity of u-PA and plasmin on the plasma membrane. Plasmin has many roles, including activation of additional uPA and other enzymes, digestion of fibrin, and digestion of components of the extracellular matrix (ECM). Digestion of the ECM surrounding a tumor removes the ECM as a physical barrier to metastasizing cells, which are then free to leave primary tumors and invade secondary sites. A review of the role of the uPA/uPAR system in cancer metastasis is provided in "The Urokinase-type Plasminogen Activator System in Cancer Metastasis: A Review", Andreasen *et al.*, Int. J. Canc. 72:1-22 (1997).

[0005] A correlation between a high level of uPA and a high rate of metastasis, and poor prognosis, has been noted in certain tumors, especially breast cancer [Quax *et al.*, J. Cell Biol. 115:191-199 (1991); Duffy *et al.*, Cancer Res. 50: 6827-6829 (1990)]. For instance, tumors of the lung [Oka *et al.*, Cancer Res. 51:3522-3525 (1991)], bladder [Hasui *et al.*, Int. J. Cancer 50:871-873 (1992)], stomach [Nekarda *et al.*, Lancet 343:117 (1994)], cervical cancer [Kobayashi *et al.*, Cancer Res. 54:6539-6548 (1994)], ovary [Kuhn et *al.*, Gynecol. Oncol. 55:401-409 (1994)], kidney [Hofmann *et al.*, Cancer 78:487-492 (1996)], brain [Bindahl *et al.*, J. Neuro-Oncol. 22:101-110 (1994)], and soft tissue sarcoma [Choong *et al.*, Int. J. Cancer (Pred. Oncol.) 69:268-272 (1996)] have exhibited a high level of uPA and/or uPA activity and a high rate of metastases. Overproduction of uPA has been reported to result in increased skeletal metastasis by prostate cancer cells *in vivo* [Achbarou *et al.*, Cancer Res. 54:2372-2377 (1994)].

[0006] Inhibition or lowering of uPA activity, or disruption/inhibition of the interaction between uPA and its receptor (uPAR) has been shown to have a positive effect on maintenance of the extracellular matrix and an inhibitory effect on metastasis [Ossowski and Reich, Cell 35:611-619 (1983); Ossowski, Cell 52:321-328 (1988); Ossowski, J. Cell Biol. 107:2437-2445 (1988); Wilhelm *et al.*, Clin. Exp. Metastasis 13:296-302 (1995); Achbarou *et al.*, Cancer Res. 54: 2372-2377 (1994); Crowley *et al.*, Proc. Natl. Acad. Sci. USA 90:5021-5025 (1993); Kook *et al.*, EMBO J. 13:3983-3991 (1994)]. The results of such experimental studies suggest that uPA-catalyzed plasminogen activation is rate-limiting for tumor progression, local tumor invasion and/or formation of distant metastasis. [Andreasen *et al.*, Int. J. Canc. 72: 1-22 (1997)].

[0007] The effects of the uPA system on cell migration and invasion are thought to be due to both a proteolytic effect of plasmin-mediated degradation of the extracellular matrix, as well as more a direct interaction of the uPA receptor with components of the extracellular matrix. Degradation of the extracellular matrix permits a metastasizing cell to invade the matrix, whereas interaction between uPA receptor and the matrix itself assists a cell in its migration. Localization of the uPA/plasmin system on the cell surface, or the leading edge of metastasizing cells, is consistent with postulated role of uPA in metastasis [Plesner *et al.*, Stem Cells 15:398-408 (1997)].

[0008] Interaction of uPAR with vitronectin, a component of the extracellular matrix, mediates cell adhesion and can be enhanced when uPAR is bound by uPA. Cell surface adhesion molecules, integrins, also appear to be involved in this adhesion function, particularly beta-1 and beta-2 integrins [Paysant *et al.*, Br. J. Haematol. 100:45-51 (1998); Simon *et al.*, Blood 88:3185-3194 (1996)]. The CD11b/CD18 integrin can associate with the uPA-uPAR complex and promote adhesion of cells bearing these receptors, *e.g.*, neutrophils, leukocytes.

[0009] The uPA/uPAR system also is involved in the establishment of new vasculature, or neovascularization.

[0010] Establishment of new vasculature is required for sustaining primary and metastatic tumor growth. Pathological neovascularization also is a characteristic of retinal disease, rubeosis iritis, proliferative vitreo retinopathy inflammatory disease, diabetic retinopathy, chronic uveitis, Fuch's heterochromic iridocyclitis, neovascular glaucoma, comeal or

optic nerve neovascularization, vascular disease, pterygium, glaucoma surgery bleb failure, hyperkeratosis, cheloid and polyp formation (see EP 451,130). Undesired angiogenesis also can occur in the following conditions or can be a result of the following activities: macular degeneration, retinopathy of prematurity, corneal graft rejection, retrolental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sogrens disease, acne rosacea, phylectenulosis, syphilis, Mycobacteria infections other than leprosy, lipid degeneration, chemical burns, bacterial or fungal ulcers, Herpes simplex or zoster infections, protozoan infections, Kaposi's sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegeners sarcoidosis, sleritis, Steven's Johnson disease, radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein or artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Bechets disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, post-laser complications, abnormal proliferation of fibrovascular tissue, hemangiomas, Osler-Wever-Rendu, solid tumors, blood borne tumors, AIDS, ocular neovascular disease, osteoarthritis, chronic inflammation, Crohn's disease, ulcerative colitis, tumors of rhabdomyosarcoma, tumors of retinoblastoma, tumors of Ewing sarcoma, tumors of neuroblastoma, tumors of osteosarcoma, leukemia, psoriasis, atherosclerosis, pemphigoid, as recited in U.S. Patent No. 5,712,291.

[0011] An antagonist of uPA/uPAR binding (EGF-like domain of uPA fused to Fc of IgG) was said to inhibit neovascularization and growth of the murine B16 melanoma. [Min *et al.*, Cancer Res. 56:2428-2433 (1996)]. Consistent with this finding is the correlation noted between microvessel density, vascular invasion and uPA levels in breast carcinomas [Hildenbrand *et al.*, Brit. J. Cancer 72:818-823 (1995)]. The known uPA inhibitor amiloride also was said to inhibit a variety of neovascularization pathologies [Glaser *et al.*, EP 451,130; Avery *et al.*, Arch. Ophthalmol. 108:1474-1476 (1990)].

[0012] There are two primary physiological inhibitors of uPA, PAI-1 and PAI-2, which are members of the serpin family of proteinase inhibitors. The binding of serpins to their cognate proteases involves a large number of interactions between amino acids of each protein, including those in the serpin reactive loop (Ser-Ala-Arg-Met-Ala (SEQ. ID. NO. 1) for PAI-1, Thr-Gly-Arg-Thr-Gly (SEQ. ID. NO. 2) for PAI-2). Introduction of exogenous PAI-2 into experimental animals was reported to inhibit the rate of lung metastasis [Evans and Lin, Amer. Surg. 61:692-697 (1995); Mueller *et al.*, Proc. Natl. Acad. Sci. USA 92:205-209 (1995)]. The ability of PAI-1 to inhibit metastasis has not yet been consistently shown. The gene for PAI-1, and means for its recombinant expression, are disclosed in Loskutoff et al., U.S. Patent No. 4,952,512. Recombinant and native human PAI-2 is disclosed in Stephens *et al.*, U.S. Patent No. 5,422,090.

[0013] The most widely studied uPA inhibitors may be within the 4-substituted benzo[b]thiophene-2-carboxamidine class of inhibitors, of which B428 (4-iodo-benzo[b]thiophene-2-carboxamidine) and B623 are members [Towle *et al.*, Cancer Res. 53:2553-2559 (1993); Bridges *et al.*, Bioorg. Med. Chem. 1:403-410 (1993); Bridges *et al.*, U.S. Patent No. 5,340,833]. Infusion of B428 in experimental rats inoculated with tumor cells was said to inhibit uPAR gene expression, decrease the primary tumor volume and decrease metastases [Xing *et al.*, Cancer Res. 57:3585-3593 (1997)]. Daily intraperitoneal treatment of mice bearing tumors with B428 or B623 was said to block metastasis to muscle and fat, but did not inhibit tumor-induced angiogenesis or reduce the rate of spontaneous lung metastasis. In fact, B623 enhanced the formation of lung metastasis (Alonso *et al.*, Breast Cancer Res. Treat. 40:209-223 (1996)]. Infusion of B428 in a syngeneic model of rat prostate cancer also lead to a decrease in primary tumor volume and tumor weight, and a decrease in metastasis [Rabbani *et al.*, Int. J. Cancer 63:840-845 (1995)].

[0014] Other known inhibitors of uPA include p-aminobenzamidine, which is a competitive inhibitor of uPA, and amiloride. Both compounds have been shown to reduce tumor size in experimental animals [Jankan *et al.*, Cancer Res. 57:559-563 (1997); Billstrom *et al.*, Int. J. Cancer 61:542-547 (1995)]. Recently, epigallo-cathecin-3 gallate (EGCG), a polyphenol found in green tea, was reported to bind uPA and inhibit its activity [Jankun *et al.*, Nature 387:561 (1997)]. Those researchers concluded EGCG is a weaker inhibitor of uPA than amiloride, but suggested EGCG can be consumed in much higher doses than amiloride without toxic effect. A competitive inhibitor of uPA, α-N-benzylsulfonyl-p-aminophenylalanine, is disclosed by Pye *et al.* in U.S. Patent No. 4,165,258.

[0015] Other approaches at inhibiting the uPA/uPAR system include development of a bifunctional hybrid molecule consisting of the uPAR-binding domain of uPA and PAI-2, which is said to inhibit uPA and bind uPAR *in vitro* [Ballance *et al.*, Eur. J. Biochem. 207:177-183 (1992)]. Antagonists of uPAR also have been studied [Doyle and Rosenberg, U. S. Patent No. 5,656,726; Min *et al.*, Cancer Res. 56:2428-2433 (1996)], as have antisense oligonucleotides complementary to uPA [Wilhelm *et al.*, Clin. Exp. Metast. 13:296-302 (1995); Iversen and Scholar, U.S. Patent No. 5,552,390]. Antibodies directed against uPAR, and said to inhibit the binding of uPA to UPAR, are disclosed by Dano *et al.* in U.S. Patent No. 5,519,120. Small molecules said to inhibit urokinase, along with a variety of other serine proteases, include those disclosed by Abe *et al.* in U.S. Patent No. 5,508,385 and U.S. Patent No. 5,153,176, and by Takano *et al.* in J. Pharmacol. Exp. Therapeut. 271:1027-1033 (1994).

[0016] Compounds have been developed to directly inhibit the binding of u-PA to uPAR (Crowley *et al.*, Proc. Natl. Acad. Sci. USA 90:5021-5025 (1993); Goodson *et al.*, Proc. Natl. Acad. Sci. USA 91:7129-7133 (1994); Kobayashi *et al.*, Brit. J. Cancer 67:537-544 (1993), and Int. J. Cancer 57:727-73f3 (1994), and J. Biol. Chem. 270:8361-8366 (1995);

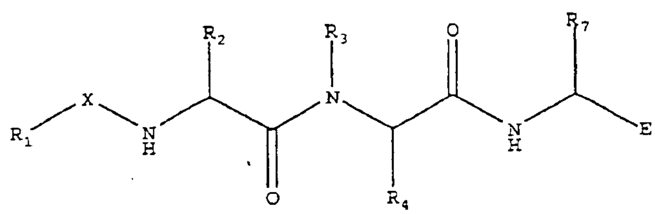
Lu *et al.*, FEBS Lett. 356:56-59 (1994) and FEBS Lett. 380:21-24 (1996)].

[0017] Additionally, pro-hepatocyte growth factor (HGF), a cell migration stimulating protein, is a substrate of uPA [Naldinie *et al.*, EMBO J. 11:4825-4833 (1992)]. Direct cleavage of a 66kDa extracellular matrix protein and fibronectin by uPA also has been reported, which suggests a more direct role for uPA in facilitating cell migration [Quigley et al., Proc. Natl. Acad. Sci. 84:2776-2780 (1987)]. Thus, inhibition of uPA may affect these activities, as well.

Summary of the Invention

[0018] The present invention is directed to novel pepitidic noncovalent urokinase inhibitors. The compounds have an arginine mimic at P1. These compounds have activity as potent inhibitors of urokinase and thereby are useful in decreasing its deleterious effects. Compounds of the present invention are active in inhibiting blood vessel formation, particularly that related to a pathologic process.

[0019] Thus in one aspect, the present invention is directed to compounds of the formula (I):



(I)

wherein:

(a) X is selected from the group consisting of $-S(O)_2-$, $-N(R')-S(O)_2-$, -(C=O)-, -OC(=O)-, -NH-C(=O)-, -P(O) (R')-, and a direct link, wherein R' is independently hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms, with the proviso that when X is -P(O) (R')-, then R' is not hydrogen;

(b) R_1 is selected from the group consisting of:

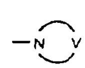
(1) alkyl of 1 to about 12 carbon atoms which is optionally substituted with Y_1 and/or Y_2,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 3 to about 8 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,

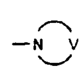
(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,

(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2, including,

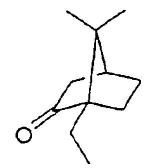


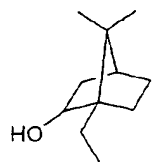
wherein

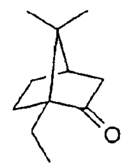


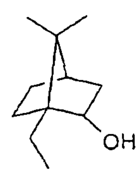
is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is $-CH_2-$, -O-, -S(=O)-, $-S(O)_2-$ or -S-, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3,

(6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cycloalkyl of about 3 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,
(8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,
(9) aralkyl of about 7 to about 15 carbon atoms which is optionally substituted on the alkyl chain with hydroxy or halogen and which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,
(10) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, which is optionally substituted on the alkyl chain with hydroxy or halogen and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(11) aralkenyl of about 8 to about 16 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,
(12) heteroaralkenyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3,

(13)  ,

(14)  ,

(15)  ,

(16)  ,

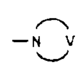
(17) fused carbocyclic alkyl of about 9 to about 15 carbon atoms;
(18) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,
(19) perfluoroaryl of about 6 to about 14 carbon atoms,
(20) perfluoroaralkyl of about 7 to about 15 carbon atoms, and

(21) hydrogen when X is a direct link;
wherein each Y_1, Y_2, and Y_3 is independently selected and is

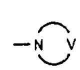
(i) selected from the group consisting of halogen, cyano, nitro, tetrazolyl, guanidino, amidino, methylguanidino, $-CF_3$, $-CF_2CF_3$, $-CH(CF_3)_2$, $-C(OH)(CF_3)_2$, $-OCF_3$, $-OCF_2H$, $-OCF_2CF_3$, $-OC(O)NH_2$, $-OC(O)NHZ_1$, $-OC(O)NZ_1Z_2$, $-NHC(O)Z_1$, $-NHC(O)NH_2$, $-NHC(O)NZ_1$, $-HC(O)NZ_1Z_2$, $-C(O)OH$, $-C(O)OZ_1$, $-C(O)NH_2$, $-C(O)NHZ_1$, $-C(O)NZ_1Z_2$, $-P(O)_3H_2$, $-P(O)_3(Z_1)_2$, $-S(O)_3H$, $-S(O)_mZ_1$, $-Z_1$, $-OZ_1$, $-OH$, $-NH_2$, $-NHZ_1$, $-NZ_1Z_2$, -N-morpholino, and $-S(O)_m(CF_2)_qCF_3$, wherein m is 0, 1 or 2, q is an integer from 0 to 5, and Z_1 and Z_2 are independently selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms, or
(ii) Y_1 and Y_2 are selected together to be $-O[C(Z_3)(Z_4)]_rO-$ or $-O[C(Z_3)(Z_4)]_{r+1}-$, wherein r is an integer from 1 to 4 and Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms;

(c) R_2 is selected from the group consisting of $-CH_3$, $-C_2H_5$, $-(CH_2)_2OH$, $-(CH_2)_2OA_1$, $-CH(R_5)OH$, $-CH(R_5)OA_1$ and $-CH_2NH-X'-R_6$ wherein A_1 is $-C(=O)OR_6$, $-C(=O)R_6$ or $-C(=O)NR_5R_6$; X' is selected from the group consisting of $-S(O)_2-$, $-S(O)_2-N(R'')-$, $-(C=O)-$, $-C(=O)-O-$, $-C(=O)-NH-$, $-P(O)(R'')-$, and a direct link, wherein R" is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms with the proviso that when X' is $-P(O)(R'')-$, then R" is not hydrogen; R_5 is selected from the group consisting of:

(1) alkyl of 1 to about 4 carbon atoms, optionally substituted with Y_1 and/or Y_2;
(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 6 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(3) cycloalkyl of 3 to about 6 carbon atoms, which is optionally mono-, di-, or trisubstituted on the ring with Y_1, Y_2, and/or Y_3;
(4) heterocycloalkyl of 4 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(5) heterocyclo of 4 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2, including



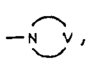
wherein



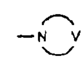
is a 5 to 6 member heterocycle having 3 to 5 ring carbon atoms, where V is $-CH_2-$, $-O-$, $-S(=O)-$, $-S(O)_2-$ or $-S-$, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3,
(6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cycloalkyl of 3 to about 6 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(7) phenyl which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3;
(8) heteroaryl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,
(9) alkyl of 1 to about 4 carbon atoms which is substituted with phenyl and which is optionally mono-, di-, or tri-substituted on the phenyl ring with Y_1, Y_2, and/or Y_3,
(10) heteroaralkyl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted

on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(11) aralkenyl of about 8 to about 12 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,
(12) heteroaralkenyl of 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3, and
(13) hydrogen; and R_6 is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms, optionally substituted with Y_1 and/or Y_2,
(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or trisubstituted on the ring with Y_1, Y_2, and/or Y_3,
(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2, including



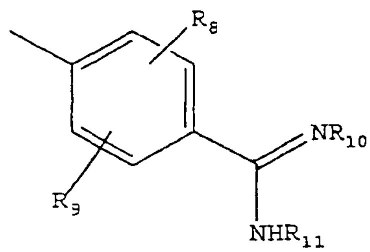
wherein



is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is $-CH_2-$, -O-, -S(=O)-, $-S(O)_2-$ or -S-, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3,
(6) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,
(7) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,
(8) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,
(9) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3, and
(10) hydrogen, with the proviso that R6 is not hydrogen when A_1 is $-C(=O)OR_6$;

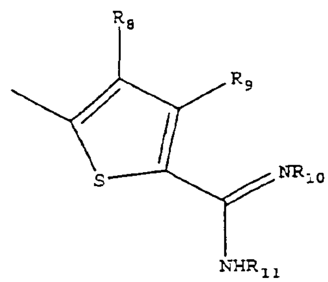
(d) R_3 is selected from H or methyl, or R_3 and R_4 are selected together as set forth in (f);
(e) R_4 is in the S configuration and is selected from the group consisting of H, $-CH_2-S-CH_3$, $-CH_2OH$, $-CH_2CN$, lower alkyl of 1 to about 3 carbon atoms, $-CH_2C{\equiv}CH$, $-CH_2CH{=}CH_2$ and $-CH{=}CH_2$ or R_3 and R_4 are selected together as set forth in (f);
(f) alternatively, R_3 and R_4 are selected together to be in the S configuration to give a group at P2 selected from the group consisting of prolyl, pipecolyl, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, 3,4-methanoprolyl, and 3,4-dehydroprolyl;
(g) R_7 is hydrogen or alkyl of 1 to about 4 carbon atoms; and
(h) E is selected from

(1)



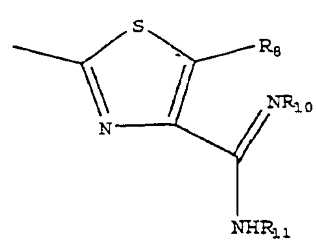
;

(2)



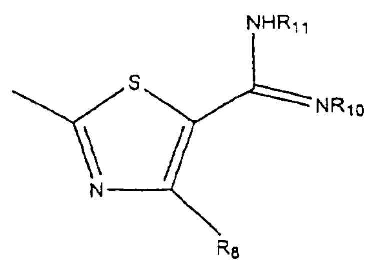
;

(3)

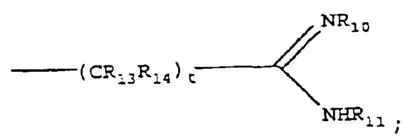


;

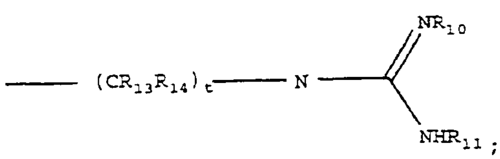
(4)



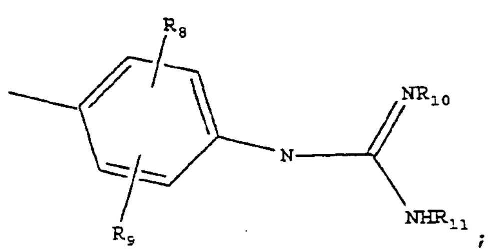
(5)



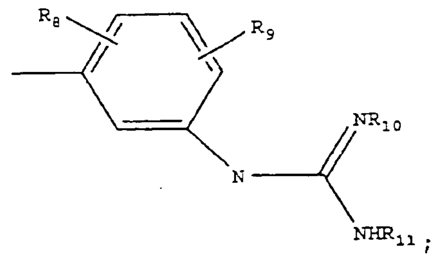
(6)



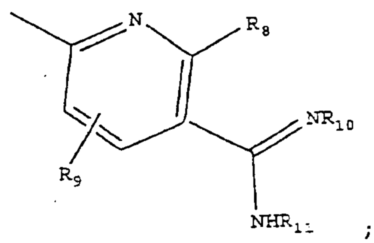
(7)



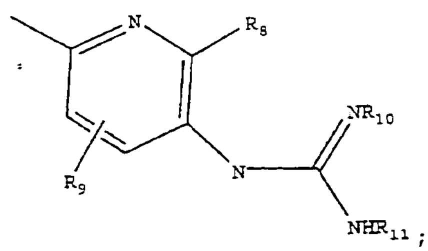
(8)



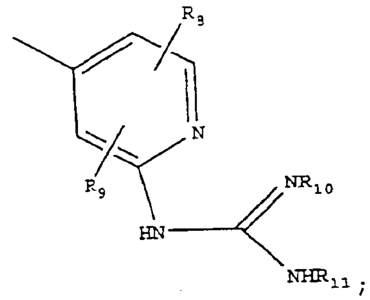
(9)



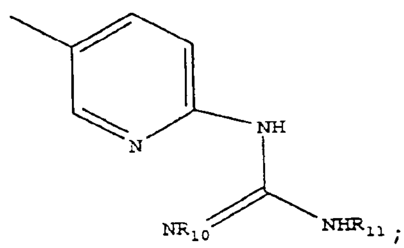
(10)



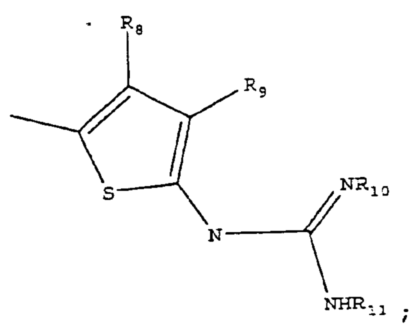
(11)



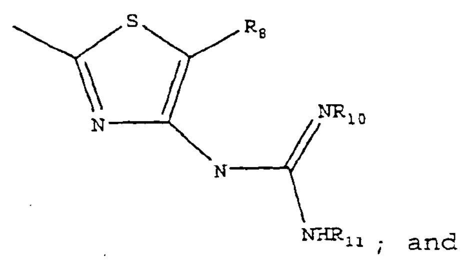
(12)



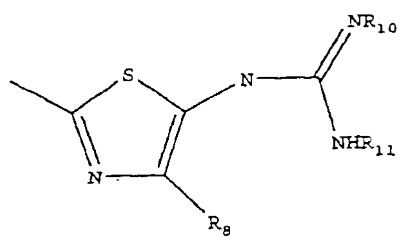
(13)



(14)

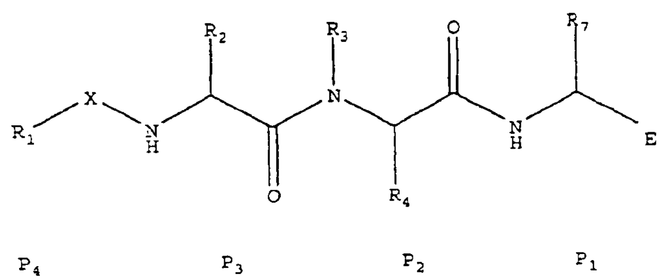


(15)

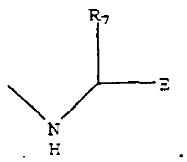


wherein R_8 and R_9 are independently selected from hydrogen, hydroxy, halogen, alkyl of 1 to about 4 carbon atoms, alkyl of 1 to about 4 carbon atoms substituted with alkoxy of 1 to about 4 carbon atoms, alkoxy of 1 to about 6 carbon atoms, and trifluoromethyl; R_{10} and R_{11} are independently hydrogen, hydroxy, alkoxy of 1 to about 3 carbon atoms, trihydrocarbylsilyl of 3 to about 16 carbon atoms, alkyl of 1 to about 3 carbon atoms or -C(=O)R_{12}; R_{12} is hydrogen, alkyl of 1 to about 6 carbon atoms, alkoxy of 1 to about 6 carbon atoms or $(CF_2)_jCF_3$ wherein j is 0, 1, 2 or 3 with the proviso that R_{10} and R_{11} are not both hydroxy or alkoxy; each of R_{13} and R_{14} is independently selected from hydrogen or lower alkyl of 1 to about 3 carbon atoms; and t is an integer from 0 to 6; and pharmaceutically acceptable salts thereof.

[0020] The compounds of the present invention can be divided into parts termed P_1, P_2, P_3 and P_4 as shown in the following formula Ia:

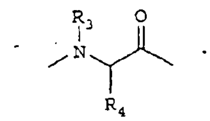


wherein X, R_1, R_2, R_3, R_4, R_7 and E are as defined in connection with formula (I). Thus, the portion of a compound of formula (I) referred to as P_1 or P1 is the moiety

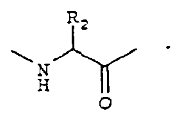


(Ia)

[0021] The portion of a compound of formula (I) referred to as P_2 or P2 is the moiety



[0022] The portion of a compound of formula (I) referred to as P_3 or P3 is the moiety



[0023] Among other factors, the present invention is based on our finding that the novel compounds of our invention are active as inhibitors of urokinase. Compounds of the present invention exhibit activity in inhibiting angiogenesis.

[0024] In another aspect, the present invention is directed to pharmaceutical compositions comprising a therapeutically effective amount of a compound of the present invention and a pharmaceutically acceptable carrier.

[0025] In yet another aspect, the present invention is directed to methods of using the compounds and pharmaceutical compositions of the present invention for inhibition of urokinase.

Definitions

[0026] In accordance with the present invention and as used herein, the following terms are defined to have following meanings, unless explicitly stated otherwise:

[0027] The term "alkenyl" refers to unsaturated aliphatic groups having at least one double bond.

[0028] The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched-chain and cyclic (including polycyclic) groups.

[0029] The terms "alkoxy" and "alkoxyl" refer to a group having the formula, R-O-, wherein R is an alkyl group.

[0030] The term "alkoxycarbonyl" refers to -C(O)OR wherein R is alkyl.

[0031] The term "aralkenyl" refers to an alkenyl group substituted with an aryl group. Preferably the alkenyl group has from 2 to about 6 carbon atoms.

[0032] The term "aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups include benzyl, phenethyl, and the like, all of which may be optionally substituted. Preferably the alkyl group has from 1 to about 6 carbon atoms.

[0033] The term "aryl" refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes a carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted.

[0034] The term "aryloxy" refers to a group having the formula, R-O-, wherein R is an aryl group.

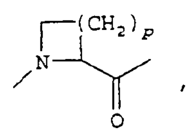
[0035] The term "aralkoxy" refers to a group having the formula, R-O-, wherein R is an aralkyl group.

[0036] The term "amino acid" refers to both natural, unnatural amino acids in their D and L stereo isomers if their structures allow such stereoisomeric forms, and their analogs. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-ami-

nopimelic acid, 2,4 diaminoisobutyric acid, demosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, hydroxylysine, allohydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, alloisoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, norvaline, norleucine, ornithine and pipecolic acid. Amino acid analogs include the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S- (carboxymethyl)-cysteine sulfone..

[0037] The term "amino acid analog" refers to an amino acid wherein either the C-terminal carboxy group, the N-terminal amino group or side-chain functional group has been chemically modified to another functional group. For example, aspartic acid-(beta-methyl ester) is an amino acid analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine.

[0038] The term "amino acid residue" refers to radicals having the structure: (1) -C(O)-R-NH-, wherein R typically is -CH(R')-, wherein R' is H or a carbon containing substituent; or (2)



wherein p is 1, 2 or 3 representing the azetidinecarboxylic acid, proline or pipecolic acid residues, respectively.

[0039] "Biaryl" refers to phenyl substituted by carbocyclic or heterocyclic aryl as defined herein, ortho, meta or para to the point of attachment of the phenyl ring.

[0040] "Brine" refers to an aqueous saturated solution of sodium chloride.

[0041] "Carbocyclic aryl" refers to aromatic groups wherein the ring atoms on the aromatic ring are carbon atoms. Carbocyclic aryl groups include monocyclic carbocyclic aryl groups and naphthyl groups, all of which may be optionally substituted. Suitable carbocyclic aryl groups include phenyl and naphthyl. Suitable substituted carbocyclic aryl groups include indene and phenyl substituted by one to two substituents such being advantageously lower alkyl, hydroxy, lower alkoxy, lower alkoxycarbonyl, halogen, trifluoromethyl, difluoromethyl, nitro, and cyano. Substituted naphthyl refers to naphthyl, more preferably 1- or 2-naphthyl, substituted by Y_1, Y_2 and/or Y_3 as defined in connection with formula (I) hereinabove.

[0042] "Cycloalkenyl" refers to a cyclic alkenyl group. Suitable cycloalkenyl groups include, for example, cyclopentenyl and cyclohexenyl.

[0043] "Cycloalkyl" refers to a cyclic alkyl group having at least one ring and includes polycyclic groups, including fused ring cyclic alkyl groups. Suitable cycloalkyl groups include, for example, cyclohexyl, cyclopropyl, cyclopentyl, and cycloheptyl.

[0044] "Cyclohexylmethyl" refers to a cyclohexyl group attached to CH_2.

[0045] "Fused carbocyclic" refers to a multicyclic fused carbocyclic ring having both aromatic and non-aromatic rings. Suitable fused carbocyclic rings include fluorenyl, tetralin and the like.

[0046] "Fused carbocyclic alkyl" refers to an alkyl group substituted with a fused carbocyclic ring moiety, preferably a multicyclic fused carbocyclic ring including both aromatic and non-aromatic rings. Suitable fused carbocyclic alkyl groups include fluorenylmethyl, and the like.

[0047] The term "halogen" refers to fluorine, chlorine, bromine and iodine.

[0048] "Heteroaralkenyl" refers to an alkenyl group substituted with a heteroaryl, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Preferably the alkenyl group has from 2 to about 6 carbon atoms.

[0049] "Heteroaralkyl" refers to an alkyl group substituted with a heteroaryl, such as picolyl, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Preferably the alkyl group has from 1 to about 6 carbon atoms.

[0050] "Heteroaryl" refers to aromatic groups having from 1 to 14 carbon atoms and the remainder of the ring atoms are heteroatoms, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Suitable heteroatoms include oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, and suitable heterocyclic aryls include furanyl, thienyl, pyridyl, pyrrolyl, pyrimidyl, pyrazinyl, imidazolyl, and the like.

[0051] "Heterocyclo" refers to a reduced heterocyclic ring system comprised of carbon, nitrogen, oxygen and/or sulfur atoms, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems.

[0052] "Heterocycloalkyl" refers to an alkyl group substituted with a heterocyclo group, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics", 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Preferably the alkyl group has from about 1 to about 6 carbon atoms.

[0053] The term "lower" referred to herein in connection with organic radicals or groups defines such radicals or groups with one and up to and including 5 carbon atoms, preferably up to and including 4 carbon atoms, and advantageously one or two carbon atoms. Such radicals or groups may be straight chain or branched chain.

[0054] "Perfluoroalkyl" refers to an alkyl group which has every hydrogen replaced with fluorine.

[0055] "Perfluoroaryl" refers to an aryl group which has every hydrogen replaced with fluorine.

[0056] "Perfluoroarylalkyl" refers to an aralkyl group in which every hydrogen on the aryl moiety is replaced with fluorine.

[0057] "Pharmaceutically acceptable salt" includes salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid. In practice the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention.

[0058] "AcN" or "MeCN" refers to acetonitrile.

[0059] "AIBN" refers to 2, 2'-azobisisobutyronitrile.

[0060] "Bn" refers to benzyl.

[0061] "Boc" refers to t-butoxycarbonyl.

[0062] "Boc_2O" refers to Boc anhydride (di-tert-butyl-carbonate).

[0063] "$BzlSO_2$" refers to benzylsulfonyl.

[0064] "Cbz" or "CBz" refers to benzyloxycarbonyl.

[0065] "$CsCo_3$" refers to cesium carbonate.

[0066] "DCA" refers to dichloroacetic acid.

[0067] "DCC" refers to N,N'-dicyclohexylcarbodiimide.

[0068] "DCM" refers to dichloromethane.

[0069] "DIEA" refers to diisopropylethylamine.

[0070] "DMF" refers to N,N-dimethylformamide.

[0071] "DMSO" refers to dimethyl sulfoxide.

[0072] "DMAP" refers to 4-N,N-dimethylaminopyridine.

[0073] "EDC" refers to 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride salt.

[0074] "Et_3N" or "TEA" refers to triethylamine.

[0075] "EtOAc" refers to ethyl acetate.

[0076] "EtOH" refers to ethanol.

[0077] "HATU" refers to O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluromium hexafluorophosphate.

[0078] "HBTU" refers to 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

[0079] "HCl" refers to hydrochloric acid.

[0080] "HOAc" refers to acetic acid.

[0081] "HOAt" or "HOAT" refers to 1-hydroxy-7-azabenzotriazole.

[0082] "HOBt" refers to 1-hydroxybenzotriazole monohydrate.

[0083] "i-BuOCOCl" refers to isobutylchloroformate.

[0084] "HPLC" refers to high pressure liquid chromatography.

[0085] "$LiAlH_4$" refers to lithium aluminum hydride.

[0086] "$LiAlH_2(OEt)_2$" refers to lithium aluminum hydride diethoxide.

[0087] "Me" refers to methyl.

[0088] "MeOH" refers to methanol.

[0089] "NMM" refers to N-methylmorpholine.

[0090] "$PhB(OH)_2$" refers to phenylboronic acid.

[0091] "Ph_3P" or "PPh_3" refers to triphenylphospine.

[0092] "PyBOP" refers to benzotriazole-ly-oxy-tris-pyrrolidinophosphonium hexafluorophosphate.

[0093] "RP-HPLC" refers to reverse phase high pressure liquid chromatography.

[0094] "TFA" refers to trifluoroacetic acid.

[0095] "THF" refers to tetrahydrofuran.

[0096] "TLC" refers to thin layer chromatography.

Brief Description Of The Drawings

[0097]

Figure 1 depicts a reaction scheme for solution phase synthesis of an intermediate useful in synthesizing a compound of the present invention. Compound **1-1** is N-α-Cbz-D-serine (O-t-butyl), compound **1-2** is alanine methyl ester, hydrochloride salt. In this figure, "i" through "iv" are defined as i) EDC, 1-hydroxybenzotriazole and acetonitrile, diisopropylethylamine to give Cbz-D-Ser (O-t-butyl)-Ala-OMe; (ii) ethanol/acetic acid/water (4:1:1), 10% Pd on carbon, 45 psi H_2 for 2 hours, 95% yield after work-up; iii) acetonitrile, benzenesulfonyl chloride, diisopropylethylamine, 43% yield after work-up; and iv) methanol, 1.0 M lithium hydroxide, acidification on DOWEX ion exchange resin, eluting with methanol/water, 95% yield after work-up. See also Examples 60 to 62.

Figure 2 depicts a reaction scheme for a solution phase synthetic route which may be used to prepare an intermediate useful in the preparation of a compound of the present invention. In this figure, "i" through "iii" are defined as:

i) isobutyl chloroformate, sodium carbonate, water, 99.5% yield after workup; ii) alanine t-butyl ester, hydrochloride salt, EDC, and hydroxybenzotriazole in acetonitrile; diisopropylethylamine, quantitative yield after workup; and

iii) TFA, DCM, quantitative yield after workup. See also Examples 74 to 76.

Figure 3 depicts a reaction scheme for the synthesis of intermediates which may be used in the preparation of compounds of the present invention.- In this figure, "i" through "xi" are defined as follows: i) CuCN, DMF, reflux (4 hours); ii) EtOAc, 10% aqueous NaCN; iii) N-bromosuccinimide, 2,2'-azobisisobutyronitrile, CCl_4, reflux (5 hours); iv) NaN_3, DMF; v) triphenylphosphine, THF, water, O C, stirring (10 hours); vi) K_2CO_3, Boc_2O, water, dioxane; vii) hydroxylamine HCl, NMM, MeOH; viii) 10% Pd/C, MeOH, 45 psi H_2 (10 hours); ix) 4M HCl in dioxane; x) $CsCO_3$, iodopropane in DMF; and xi) 4M HCl, dioxane, 3 hours, room temperature.

Figure 4 depicts a reaction scheme for the synthesis of intermediates which may be used in the preparation of compounds of the present invention. In this figure, "i" through "iv" are defined as follows: i) NaN_3, DMF; ii) 10% Pd/C, EtOAc, 45 psi H_2 (11 hours); iii) hydroxylamine HCl, NMM, MeOH; and iv) 10% Pd/C, MeOH, 45 psi H_2 (48 hours).

Figure 5 depicts a reaction scheme for the synthesis of intermediates which may be used in the preparation of compounds of the present invention. In this figure, "i" through "vii" are defined as follows: i) Cu(I)CN, DMF; ii) NBS, benzoylperoxide, CCl_4, 80°C (14 hours); iii) NaN_3, DMF, stirring (20 hours); iv) hydroxylamine HCl, NMM, MeOH, stirring (3 days); v) $CsCO_3$, iodopropane, DMF, 50°C (20 hours); vi) triphenylphosphine, THF, stirring (20 hours); and vii) 3M NaOH to pH 14.

Figure 6 depicts a reaction scheme for the synthesis of a compound of the present invention where R_2 is $-CH_2OA_1$ and A_1 is $-C(=O)R_6$, using as an intermediate, compound of Example 9. In this figure, "i" is defined as: i) pyridine, R_6COCl.

Figure 7 depicts a reaction scheme for the synthesis of certain compounds of the present invention. In this figure, "i" through "vi" are defined as follows: i) trifluoracetic anhydride, 0°C, stir overnight; ice, CH_2Cl_2, Na_2SO_4; ii) Pd/C (10%) in MeOH (overnight); iii) N-N'-di-Boc-N"-trifluoromethanesulfonyl-guanidine, TEA, CH_2Cl_2, 6 hours; HCl, brine, Na_2SO_2; column chromatography (CH_2Cl_2/MeOH 99:1); iv) potassium carbonate, H_2O/MeOH (2:15), overnight; CH_2Cl_2/MeOH (9:1), Na_2SO_4; v) benzylsulfonyl-D-serine-L-alanine carboxylate, HATU, HOAT, DIEA, Acetonitrile, overnight; EtOAc, HCl, $NaHCO_3$, brine; HPLC (CH_3CN, H_2O, 0.1% TFA) ; vi) CH_2Cl_2/TFA (1:1), 90 minutes; HPLC (CH_3CN, H_2O, 0.1% TFA).

Figure 8 depicts the reaction scheme for the synthesis of certain compounds of the present invention. In this figure "i" throught "v" are defined as: i) trifluoroacetic anyhydride, stir overnight; ice, CH_2Cl_2, Na_2SO_4; ii) Pd/C (10%) in MeOH (overnight); iii) N-N'-Boc-N"-trifluoromethanesulfonylguanidine, TEA, CH_2Cl_2, 24 hours; HCl, brine, Na_2SO_4; column chromatography (CH_2Cl_2/MeOH 98:2); iv) potassium carbonate, H_2O/MeOH (1:1), overnight; H_2O, CH_2Cl_2/MeOH (9:1), Na_2SO_4; and v) benzylsulfonyl-D-serine-L-alanine carboxylate, HATU, HOAT, DIEA, in acetonitrile, room temperature overnight; EtOAc, HCl, $NaHCO_3$, brine; CH_2Cl_2/TFA (1:1), room temperature, 2 hours, HPLC (CH_3CN, H_2O, 0.1% TFA).

Figure 9 depicts a reaction scheme for the synthesis of a compound of the present invention. In this figure, "i" through "vi" are defined as follows: i) trifluoroacetic anhydride, 0°C, one hour; ii) KNO_3, -20°C, stir overnight; CH_2Cl_2, Na_2SO_4, column chromatography; iii) HCl in MeOH, 0°C, $SnCl_2$, stir 30 minutes, $NaHCO_3$, CH_2Cl_2, Na_2SO_4; iv) N_1N'-di-Boc-N"-trifluoromethanesulfonyl-guanidine, TEA, CH_2Cl_3, stirring 24 hours; HCl (1M), brine, Na_2SO_4, column chromatography; v) K_2CO_3, H_2O/MeOH (1:1), stir overnight; H_2O, CH_2Cl_2/MeOH (95:5), Na_2SO_4; and vi) benzylsulfonyl-D-serine-L-alanine carboxylate, HATU, HOAT, DIEA, AcN, stir overnight; EtOAc, HCl (1M),

aqueous $NaHCO_3$, brine, Na_2SO_4; HPLC.

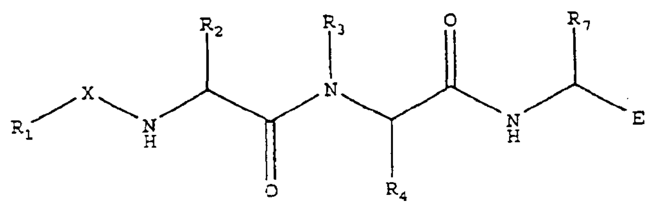
Figures 10A to 10F depict certain preferred compounds of the present invention.

Figure 11 depits a reaction scheme for the synthesis of a compound of the present invention. In this figure, "i" through "xii" are defined as follows: i) CCl_4, N-bromosuccinimide; N_2, AIBN, stirring, flash chromatography; ii) DMF, NaN_3, stir overnight, diethylether, water, brine, $MgSO_4$, filter; (iii) MeOH, TEA, 65°C, 4 hours; EtOAc, H_2O, brine, $MgSO_4$; iv) THF/water, Ph_3P, stir overnight; 1N HCl, H_2O, DCM, ph~9; v) dioxane/water, K_2CO_3, Boc_2O, stir overnight; EtOAc, aqueous $NAHCO_3$, brine, Na_2SO_4, flash column chromatography; vi) DMF, 2-iodopropane, $CsCO_3$; EtOAc, aqueous $NaHCO_3$, Na_2SO_4, flash column chromatography; vii) dioxane, 4N HCl in dioxane, solvent removal; viii) AcN, DIEA; Boc-alanine, EDC, HOBt, stir overnight; EtOAc, aqueous $NaHCO_3$, brine, Na_2SO_4, flash column chromatography; ix) dioxane, 4N HCl in dioxane, remove solvent, x) AcN, DIEA, $BnSO_2$-dSer(tBu)-OH, EDC, HOBt, stir overnight; EtOAc, aqueous $NaHCO_3$, brine, Na_2SO_4, RP-HPLC; xc) DCM, TFA; RP-HPLC; and xii) H_2O, HOAc, Zn dust; RP-HPLC.

Detailed Description of the Invention

1. Preferred Compounds

[0098] Compounds of the present invention have the formula:



(I)

wherein:

(a) X is selected from the group consisting of $-S(O)_2-$, $-N(R')-S(O)_2-$, -(C=O)-, -OC(=O)-, -NH-C(=O)-, -P(O)(R')-, and a direct link, wherein R' is independently hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms, with the proviso that when X is -P(O)(R')-, then R' is not hydrogen;

(b) R_1 is selected from the group consisting of:

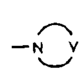
(1) alkyl of 1 to about 12 carbon atoms which is optionally substituted with Y_1 and/or Y_2,

(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of about 3 to about 8 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,

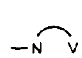
(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,

(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,

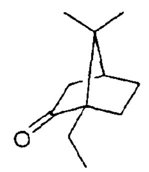
(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2, including,

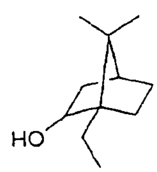


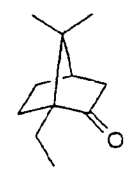
wherein

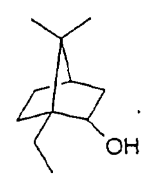


is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is $-CH_2-$, -O-, -S(=O)-, $-S(O)_2-$ or -S-, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3,
(6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cycloalkyl of 3 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(7) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,
(8) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,
(9) aralkyl of about 7 to about 15 carbon atoms which is optionally substituted in the alkyl chain with hydroxy or halogen and which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,
(10) heteroaralkyl of about 5 to 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, which is optionally substituted on the alkyl chain with hydroxy or halogen and which is optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(11) aralkenyl of about 8 to about 16 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,
(12) heteroaralkenyl of about 5 to 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3,

(13)  ,

(14)  ,

(15)  ,

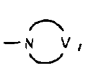
(16)  ,

(17) fused carbocyclic alkyl of about 9 to about 15 carbon atoms;
(18) difluoromethyl or perfluoroalkyl of 1 to about 12 carbon atoms,
(19) perfluoroaryl of about 6 to about 14 carbon atoms,
(20) perfluoroaralkyl of about 7 to about 15 carbon atoms, and
(21) hydrogen when X is a direct link;
wherein each Y_1, Y_2, and Y_3 is independently selected and is

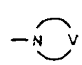
(i) selected from the group consisting of halogen, cyano, nitro, tetrazolyl, guanidino, amidino, methylguanidino, $-CF_3$, $-CF_2CF_3$, $-CH(CF_3)_2$, $-C(OH)(CF_3)_2$, $-OCF_3$, $-OCF_2H$, $-OCF_2CF_3$, $-OC(O)NH_2$, $-OC(O)NHZ_1$, $-OC(O)NZ_1Z_2$, $-NHC(O)Z_1$, $-NHC(O)NH_2$, $-NHC(O)NZ_1$, $-NHC(O)NZ_1Z_2$, $-C(O)OH$, $-C(O)OZ_1$, $-C(O)NH_2$, $-C(O)NHZ_1$, $-C(O)NZ_1Z_2$, $-P(O)_3H_2$, $-P(O)_3(Z_1)_2$, $-S(O)_3H$, $-S(O)_mZ_1$, $-Z_1$, $-OZ_1$, $-OH$, $-NH_2$, $-NHZ_1$, $-NZ_1Z_2$, -N-morpholino, and $-S(O)_m(CF_2)_qCF_3$, wherein m is 0, 1 or 2, q is an integer from 0 to 5, and Z_1 and Z_2 are independently selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms, or
(ii) Y_1 and Y_2 are selected together to be $-O[C(Z_3)(Z_4)]_rO-$ or $-O[C(Z_3)(Z_4)]_{r+1}-$, wherein r is an integer from 1 to 4 and Z_3 and Z_4 are independently selected from the group consisting of hydrogen, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, heteroaryl of about 5 to about 14 ring atoms, aralkyl of about 7 to about 15 carbon atoms, and heteroaralkyl of about 5 to about 14 ring atoms;

(c) R_2 is selected from the group consisting of $-CH_3$, $-C_2H_5$, $-(CH_2)_2OH$, $-(CH_2)_2OA_1$, $-CH(R_5)OH$, $-CH(R_5)OA_1$ and $-CH_2NH-X'-R_6$ wherein A_1 is $-C(=O)OR_6$, $-C(=O)R_6$ or $-C(=O)NR_5R_6$; X' is selected from the group consisting of $-S(O)_2-$, $-S(O)_2-N(R")-$, $-(C=O)-$, $-C(=O)-O-$, $-C(=O)-NH-$, $-P(O)(R")-$, and a direct link, wherein R" is hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms or aralkyl of about 7 to about 16 carbon atoms with the proviso that when X' is $-P(O)(R")-$, then R" is not hydrogen; R_5 is selected from the group consisting of:

(1) alkyl of 1 to about 4 carbon atoms, optionally substituted with Y_1 and/or Y_2;
(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 6 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(3) cycloalkyl of 3 to about 6 carbon atoms, which is optionally mono-, di-, or trisubstituted on the ring with Y_1, Y_2, and/or Y_3;
(4) heterocycloalkyl of 4 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(5) heterocyclo of 4 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2, including



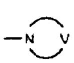
wherein



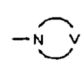
is a 5 to 6 member heterocycle having 3 to 5 ring carbon atoms, where V is $-CH_2-$, $-O-$, $-S(=O)-$, $-S(O)_2-$ or $-S-$, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3,
(6) alkenyl of 2 to about 6 carbon atoms which is optionally substituted with cycloalkyl of 3 to about 6 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(7) phenyl which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3;
(8) heteroaryl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,

(9) alkyl of 1 to about 4 carbon atoms substituted with phenyl and which is optionally mono-, di-, or tri-substituted on the phenyl ring with Y_1, Y_2, and/or Y_3,
(10) heteroaralkyl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(11) aralkenyl of about 8 to about 12 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,
(12) heteroaralkenyl of about 5 to about 6 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3, and
(13) hydrogen; and R_6 is selected from the group consisting of:

(1) alkyl of 1 to about 12 carbon atoms, optionally substituted with Y_1 and/or Y_2,
(2) alkyl of 1 to about 3 carbon atoms substituted with cycloalkyl of 3 to about 8 carbon atoms, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(3) cycloalkyl of 3 to about 15 carbon atoms, which is optionally mono-, di-, or trisubstituted on the ring with Y_1, Y_2, and/or Y_3,
(4) heterocycloalkyl of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1 or 2, which is optionally mono-, di-, or tri-substituted on the ring with Y_1, Y_2, and/or Y_3,
(5) heterocyclo of 4 to about 10 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from the group consisting of oxygen, nitrogen, and $S(O)_i$, wherein i is 0, 1, or 2, including


,

wherein



is a 5 to 7 member heterocycle having 3 to 6 ring carbon atoms, where V is $-CH_2-$, -O-, -S(=O)-, $-S(O)_2-$ or -S-, which is optionally mono-, di-, or tri-substituted on the ring carbons with Y_1, Y_2, and/or Y_3,

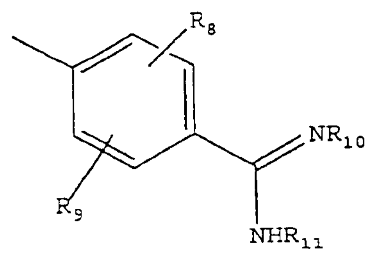
(6) aryl of about 6 to about 14 carbon atoms which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,
(7) heteroaryl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally mono-, di- or tri-substituted with Y_1, Y_2, and/or Y_3,
(8) aralkyl of about 7 to about 15 carbon atoms which is optionally mono-, di-, or tri-substituted on the aryl ring with Y_1, Y_2, and/or Y_3,
(9) heteroaralkyl of about 5 to about 14 ring atoms with the ring atoms selected from carbon and heteroatoms, wherein the heteroatoms are selected from oxygen, nitrogen, and sulfur, and which is optionally substituted on the alkyl chain with hydroxy or halogen and optionally mono-, di- or tri-substituted on the ring with Y_1, Y_2, and/or Y_3, and
(10) hydrogen with the proviso that R_6 is not hydrogen when A_1 is $-C(=O)OR_6$;

(d) R_3 is selected from H or methyl, or R_3 and R_4 are selected together as set forth in (f);
(e) R_4 is in the S configuration and is selected from the group consisting of H, $-CH_2-S-CH_3$, $-CH_2OH$, $-CH_2CN$, lower alkyl of 1 to about 3 carbon atoms, $-CH_2C{\equiv}CH$, $-CH_2CH{=}CH_2$ and $-CH{=}CH_2$ or R_3 and R_4 are selected together as set forth in (f);
(f) alternatively, R_3 and R_4 are selected together to be in the S configuration to give a group at P2 selected from the group consisting of prolyl, pipecolyl, azetidine-2-carbonyl, 4-hydroxyprolyl, 3-hydroxyprolyl, 3,4-methanoprolyl, and 3,4-dehydroprolyl;

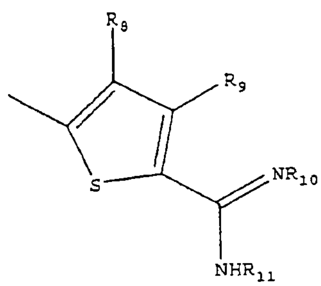
(g) R_7 is hydrogen or alkyl of 1 to about 4 carbon atoms; and

(h) E is selected from

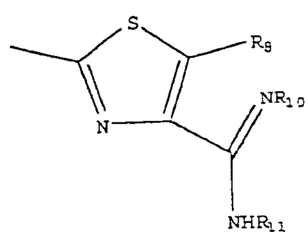
(1)

 ;

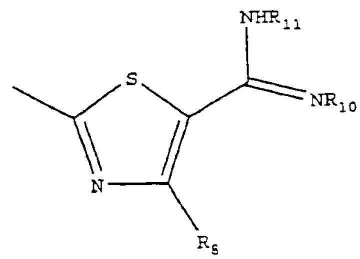
(2)

 ;

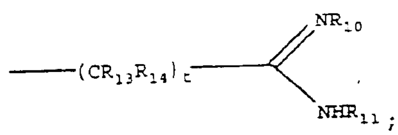
(3)

 ;

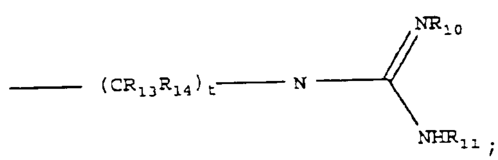
(4)



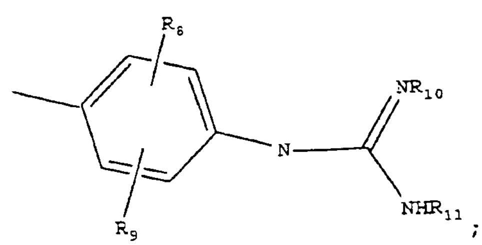
(5)



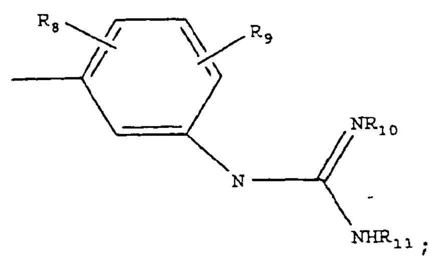
(6)



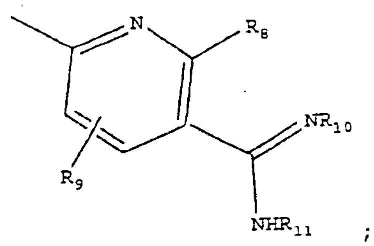
(7)



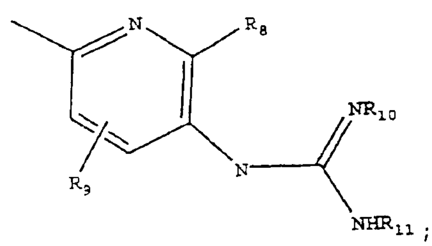
(8)



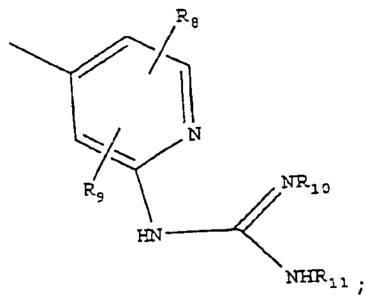
(9)



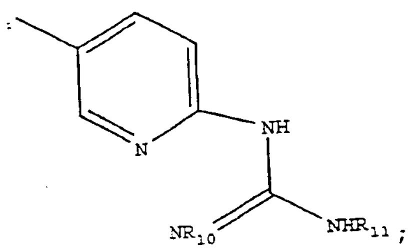
(10)



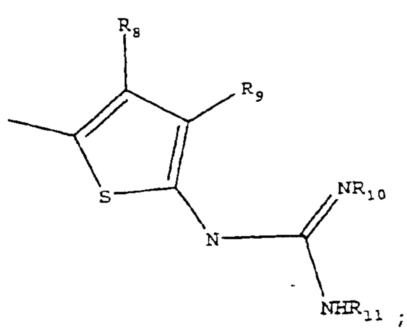
(11)



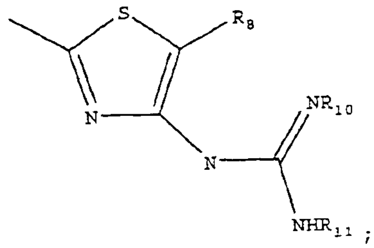
(12)



(13)

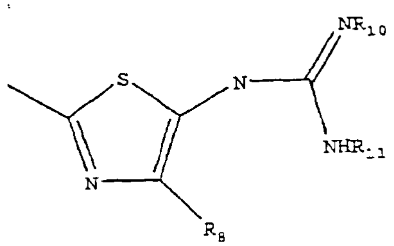


(14)



and

(15)



wherein R_8 and R_9 are independently selected from hydrogen, hydroxy, halogen, alkyl of 1 to about 4 carbon atoms, alkyl of 1 to about 4 carbon atoms substituted with alkoxy of 1 to about 4 carbon atoms, alkoxy of 1 to about 6 carbon atoms, and trifluoromethyl; R_{10} and R_{11} are independently hydrogen, hydroxy, alkoxy of 1 to about 3 carbon atoms, trihydrocarbylsilyl of 3 to about 16 carbon atoms, alkyl of 1 to about 3 carbon atoms or $-C(=O)R_{12}$; R_{12} is hydrogen, alkyl of 1 to about 6 carbon atoms, alkoxy of 1 to about 6 carbon atoms or $(CF_2)_jCF_3$ wherein j is 0, 1, 2 or 3 with the proviso that R_{10} and R_{11} are not both hydroxy or alkoxy; each of R_{13} and R_{14} is independently selected from hydrogen or lower alkyl of 1 to about 3 carbon atoms; and t is an integer from 0 to 6; and pharmaceutically acceptable salts thereof.

[0099] Preferred X groups include $-S(O)_2-$, -OC(=O)-, -NH-C(=O)-, and a direct link. Especially preferred are $-S(O)_2-$ and -OC(=O)-.

[0100] Preferred R_1 groups include alkyls, especially isobutyl, 2-ethylhexyl, methyl, n-butyl, isopropyl, cyclohexylmethyl, and cyclohexylpropyl; cycloalkyl, especially (-)menthyl, (+)menthyl, and cyclohexyl; aryls, especially naphthyl and phenyl; aralkyls, especially benzyl, 3-phenylpropyl, and 2-phenylethyl; and fused carbocyclic alkyls, especially fluorenylmethyl. Especially preferred R_1 groups include phenyl, benzyl, 2-phenylethyl, isobutyl, n-butyl and 3-phenylpropyl.

[0101] Preferred combinations of R_1-X- include phenyl-$S(O)_2$-, benzyl-$S(O)_2$-, 2-phenylethyl-$S(O)_2$-, 3-phenylpropyl-$S(O)_2$-, n-butyl-$S(O)_2$-, benzyl-OC(=O)-, and isobutyl-OC(=O)-.

[0102] Preferred R_2 groups include $-CH_3$, $-C_2H_5$, $-CH_2NH-X'-R_5$ and $-CH(R_5)OH$, wherein R_5 is hydrogen, alkyl, especially methyl, or aralkyl. Preferred chirality at the alpha carbon is R. When chiral, preferred chirality at the beta carbon is R. Preferred R_2 groups are those that define the P_3 position as d-seryl ($-CH(R_5)OH$ where R_5 is H) , (R,R) d-allothreonyl ($-CH(R_5)OH$ where R_5 is methyl), d-2-aminobutyryl, N-β-methyloxycarbonyl-d-2,3-diaminopropionyl ($-CH_2NH-X'-R_5$ where R_5 is CH_3 and X' is (-C=O)O-), N-β-(2-phenylethylcarbonyl)-d-2,3-diaminopropionyl ($-CH_2NH-X-R_5$ where R_5 is 2-phenylethyl and X' is -(C=O)-), N-β-benzyloxycarbonyl-d-2,3-diaminopropionyl ($-CH_2NH-X'-R_5$ where R_5 is benzyl and X' is -(C=O)O-) and d-alanyl($-CH_2$). Especially preferred R_2 groups are those which define P_3 as d-seryl (R_5 is H) or (R,R)d-allothreonyl (R_5 is methyl).

[0103] Alternate preferred R_2 groups include $-(CH_2)_2OA_1$ and $-CH(R_5)OA_1$, more preferably $-CH(R_5)OA_1$; preferably R_5 is H. More preferably R_2 is selected so that P_3 is defined as an acyl or carbonate ester of d-seryl. Compounds wherein R_2 is $-(CH_2)_2OA_1$ or $-CH(R_5)OA_1$ may act as prodrugs.

[0104] A preferred R_3 group, when R_3 and R_4 are not selected together, is hydrogen. A preferred R_4 group, when R_3 and R_4 are not selected together, is methyl, vinyl, allyl or propargyl. When R_3 and R_4 are selected together, prolyl, 3-hydroxy-prolyl, 4-hydroxyprolyl, 3,4-dehydroprolyl, 3,4-methanoprolyl, and azetidine-2-carbonyl- are preferred selections to define a group at P2.

[0105] Preferred R_7 groups include hydrogen.

[0106] Preferred E groups include 4-amidinophenyl, 4-guanidinophenyl, 3-amidinopropyl, and 5-(2-amidino-thienyl).

[0107] Among the compounds of the present invention, preferred compounds include those having an R_2 element that defines d-serine or d-allothreonine or an acyl or carbonate ester thereof at the P3 position of the compound and an amidinophenyl, guanidinophenyl or amidinothienyl group at P1. Especially preferred are such compounds also having either i) a hydrogen at R_3 and methyl at R_4 (P2 is alanine), or ii) having R_3 and R_4 selected together so that P2 is prolyl, azetidine-2-carbonyl, 3,4-methanoprolyl or 3,4-dehydroprolyl.

[0108] Preferred compounds of the present invention include those depicted in Figures 10A to 10F. Especially preferred are Compounds D, F, I, J, K, L. O, R, T, U, V, AE, AH, AJ, AN and AV of Figures 10A to 10F.

[0109] Also especially preferred are compounds Ax ($X=S(O)_2$, R_1=4-chlorobenzyl, $R_2=-CH_2OH$, $R_3=H$, $R_4=CH_3$, $R_7=H$ and E=4-amidinophenyl), AY ($X=SO_2$, R_1=3-chlorobenzyl, $R_2=-CH_2OH$, $R_3=H$, $R_4=CH_3$, $R_7=H$ and E=4-amidinophenyl) and AZ ($X=SO_2$, R_1=2-fluorobenzyl, $R_2=-CH_2OH$, $R_3=H$, $R_4=CH_3$, $R_7=H$ and E=4-amidinophenyl). The substitutions are to be made with respect to formula I.

2. Preparation of Preferred Compounds

[0110] Figures 1 to 5 depict synthetic schemes for synthesis of intermediates which may be used in preparation of certain compounds of the present invention.

[0111] Figure 1 depicts solution phase synthesis of intermediates useful in the preparation of compounds of the present invention. See Examples 60 to 62. See also Examples 95 to 97.

[0112] Examples 63 to 66, 67 to 70 and 71 to 73 describe solution phase syntheses of intermediates useful in the synthesis of compounds of the present invention.

[0113] Figure 2 depicts an alternate synthetic route to prepare an intermediate useful in the preparation of compounds of the present invention using solution phase synthesis. See also Examples 74 to 76.

[0114] Figure 6 depicts a reaction scheme for the preparation of a compound of the present invention having an esterified hydroxyl at P3.

[0115] Figure 7 depicts a reaction scheme for the preparation of a compound of the present invention having a 4-guanidinophenyl at P1.

[0116] Figure 8 depicts a reaction scheme for the preparation of a compound of the present invention having a 3-guanidinophenyl at P1.

[0117] Figure 9 depicts a reaction scheme for the preparation of a compound of the present invention having 2-guanidinothiophenyl at P1.

[0118] Figure 11 depicts a reaction scheme for the preparation of a compound of the present invention having a 3-amidinopyridyl at P1.

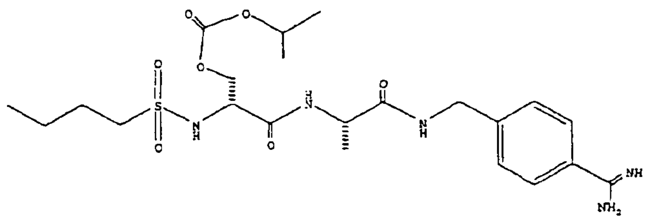
[0119] Preferred means of chemically coupling (as for example, amide bond function) include formation of a peptide bond by using conventional coupling reagents known in the art. See Bodanszky, N. Peptide Chemistry, pp. 55-73, Springer-Verlag, New York (1988) and references cited therein. The chemical coupling may be either by means of one-step or two-step coupling. In one-step coupling, the two coupling partners are coupled directly. Preferred coupling reagents for one-step coupling of the coupling partners include DCC with HOBt, EDC with HOBt, EDC with HOAt, HBTU or TBTU. In two-step coupling, an activated ester or anhydride of the C-terminal carboxy group of one coupling partner is formed prior to its coupling to the other coupling partner.

[0120] For preparation of certain compounds having hydrogenation-sensitive substituent groups, it is preferred to avoid the use of hydrogen gas with palladium on carbon. Another preferred method for preparing compounds of the present invention containing hydrogenation sensitive groups such as alkenyl or aryl moieties substituted with halogen, cyano, nitro, or $-S-Z_1$, is to use boron tris(trifluoroacetate), $B(OCOCF_3)_3$, to cleave the N^g-nitro of the arginine group. The reagent is prepared by the reaction of BBr_3 and CF_3COOH in dichloromethane at 0°C. The reagent is also commercially available. Generally, the N^g-nitro compound is treated with boron tris(trifluoroacetate) in trifluoroacetic acid at 0°C. *See, e.g.,* Fieser, M. and Fieser, L. F., Reagents for Organic Synthesis, p. 46, John Wiley & Sons, New York (1974); Pless, J., and Bauer, W. Angew. Chem., Internat. Ed., 12, 147 (1973).

[0121] In addition, another preferred reagent for selective nitro group cleavage is titanium trichloride. This reagent is commercially available. The N^g nitro compound is treated with titanium trichloride in aqueous methanol containing an ammonium acetate buffer followed by exposure of the reaction mixture to air or dimethyl sulfoxide. See, e.g., Freidinger, R.M., Hirschmann, R., and Veber, D.F., J. Org. Chem., 43, 4800 (1978).

[0122] Figure 6 depicts a reaction scheme for the synthesis of a compound of the present invention where R_2 is

$-CH_2OA_1$ and A_1 is $-C(=O)R_6$:



An intermediate such as **6-1** (the compound of Example 9) is reacted with an acid chloride R_6COCl in the presence of a base such as pyridine. Compounds where R_2 is $-(CH_2)_2OA_1$ or $-CH(R_5)OA_1$ where A_1 is $-C(=O)R_6$ may conveniently be prepared by reacting an appropriate intermediate corresponding to **6-1** with the appropriate acid chloride derivative, R_6COCl, preferably in the presence of a base such as pyridine.

[0123] Compounds where R_2 is $-(CH_2)_2OA_1$ or $-CH(R_5)OA_1$ wherein A_1 is $-C(=O)OR_6$ may be conveniently prepared by treating a corresponding compound where R_2 is $-(CH_2)_2OH$ or $-CH(R_5)OH$ with the appropriate chloroformate derivative. In the preparation of compounds having an amidino or guanidino group at P_1, it may be preferred to cap the P3 hydroxyl with the carbonate group prior to deprotecting the amidino or guanidino group. Accordingly, it is preferred to treat the corresponding intermediate with the chloroformate derivative. (See, *e.g.*, Example 8). The product is then hydrogenated and optionally treated under hydrolysis conditions to yield the product. (See, *e.g.*, Examples 16, 21, 28, 35 and 40.)

3. Selection of Preferred Compounds

[0124] According to one aspect of the present invention, preferred compounds of the present invention are selected for their potency and selectivity toward inhibition of serine proteases, especially urokinase. Such evaluations are routinely performed *in vitro,* following procedures such as those set forth in Example A. As described therein, and as generally known, a target serine protease and its substrate are combined under assay conditions permitting reaction of the protease with its substrate. The assay is performed in the absence of test compound, and in the presence of increasing concentrations of the test compound. The concentration of test compound at which 50% of the serine protease activity is inhibited by the test compound is the IC_{50} value (Inhibitory Concentration) or EC_{50} (Effective Concentration) value for that compound. Within a series or group of test compounds, those having lower IC_{50} or EC_{50} values are considered more potent inhibitors of the serine protease than those compounds having higher IC_{50} or EC_{50} values. The IC_{50} measurement is often used for more simplistic assays, whereas the EC_{50} is often used for more complicated assays, such as those employing cells. K_i is calculated from the IC_{50}.

[0125] Preferred compounds according to this aspect of the present invention have a K_i value of 100nM or less as measured in an *in vitro* assay for inhibition of urokinase activity. Especially preferred compounds have a K_i value of less than 30nM.

[0126] The test compounds also are evaluated for selectivity toward a serine protease. As described in the Examples, and as generally known, a test compound is assayed for its potency toward a panel of serine proteases and other enzymes and an IC_{50} value or EC_{50} value is determined for each test compound in each assay system. A compound that demonstrates a low IC_{50} value or EC_{50} value or corresponding low K_i value for the target enzyme, *e.g.*, urokinase, and a higher IC_{50} value or EC_{50} value for other enzymes within the test panel (*e.g.*, tissue plasminogen activator, thrombin, Factor Xa), is considered to be selective toward the target enzyme. Generally, a compound is deemed selective if its IC_{50} value or EC_{50} value (or K_i value) in the target enzyme assay is at least one order of magnitude less than the next smallest IC_{50} value or EC_{50} value measured in the selectivity panel of enzymes.

[0127] Preferred compounds of the present invention have a K_i value of 100nM or less as measured in an *in vitro* assay for inhibition of urokinase activity. Especially preferred compounds have a K_i value in the *in vitro* urokinase inhibition assay that is at least one order of magnitude smaller than the IC_{50} value measured in the *in vitro* tPA inhibition assay. Compounds having a selectivity ratio of IC_{50} tPA assay: K_i urokinase assay of greater than 100 are especially preferred.

[0128] Compounds of the present invention also are evaluated for their activity *in vivo.* The type of assay chosen for evaluation of test compounds will depend on the pathological condition to be treated or prevented by use of the compound, as well as the route of administration to be evaluated for the test compound.

[0129] For instance, to evaluate the activity of a compound of the present invention to reduce tumor growth through

inhibition of urokinase, the procedures described by Jankun *et al.* [Canc. Res. 57:559-563, 1997] to evaluate PAI-1 can be employed. Briefly, the ATCC cell lines DU145, which expresses a high level of uPA, and LnCaP, which does not express uPA, are injected into SCID mice. After tumors are established, the mice are given test compound according to a dosing regime determined from the compound's *in vitro* characteristics. The Jankun *et al.* compound was administered in water. Tumor volume measurements are taken twice a week for about five weeks. A compound is deemed active if an animal to which the compound was administered exhibited decreased tumor volume, as compared to animals receiving appropriate control compounds. Furthermore, a comparison of a compound's effect in animals injected with DU145 cells versus LnCaP cells can indicate whether the compound's effect was due to inhibition of urokinase or otherwise.

[0130] Another *in vivo* experimental model designed to evaluate the effect of p-aminobenzamidine, a purported urokinase inhibitory compound, on reducing tumor volume is described by Billström *et al.* [Int. J. Cancer 61:542-547, 1995].

[0131] To evaluate the ability of a compound of the present invention to reduce the occurrence of, or inhibit, metastasis, the procedures described by Kobayashi *et al.* [Int. J. Canc. 57:727-733d, 1994] can be employed. Briefly, a murine xenograft selected for high lung colonization potential is injected into C57B1/6 mice i.v. (experimental metastasis) or s.c. into the abdominal wall (spontaneous metastasis). Various concentrations of the compound to be tested can be admixed with the tumor cells in Matrigel prior to injection. Daily i.p. injections of the test compound are made either on days 1-6 or days 7-13 after tumor inoculation. The animals are killed about three or four weeks after tumor inoculation, and the lung tumor colonies are counted. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing and route of administration.

[0132] The activity of the compounds of the present invention toward decreasing tumor volume and metastasis can be evaluated in the model described by Rabbani *et al.* [Int. J. Cancer 63:840-845, 1995] to evaluate their inhibitor. There, Mat LyLu tumor cells over-expressing uPA were injected into the flank of Copenhagen rats. The animals were implanted with osmotic minipumps to continuously administer various doses of test compound for up to three weeks. The tumor mass and volume of experimental and control animals were evaluated during the experiment, as were metastatic growths. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing, and route of administration. Some of these authors described a related protocol in Xing *et al.* [Canc. Res. 57: 3585-3593, 1997].

[0133] To evaluate the inhibitory activity of a compound of the present invention toward neovascularization, a rabbit cornea neovascularization model can be employed. Avery *et al.* [Arch. Ophthalmol. 108:1474-1475, 1990] describe anesthetizing New Zealand albino rabbits and then making a central corneal incision and forming a radial corneal pocket. A slow release prostaglandin pellet was placed in the pocket to induce neovascularization. Test compound was administered i.p. for five days, at which time the animals were killed. The effect of the test compound is evaluated by review of periodic photographs taken of the limbus, which can be used to calculate the area of neovascular response and, therefore, limbal neovascularization. A decreased area of neovascularization as compared with appropriate controls indicates the test compound was effective at decreasing or inhibiting neovascularatization.

[0134] An angiogenesis model used to evaluate the effect of a test compound in preventing angiogenesis is described by Min *et al.* [Canc. Res. 56:2428-2433, 1996]. C57BL6 mice receive subcutaneous injections of a Matrigel mixture containing bFGF, as the angiogenesis-inducing agent, with and without test compound. After five days, the animals are killed and the Matrigel plugs, in which neovascularization can be visualized, are photographed. An experimental animal receiving Matrigel and an effective dose of test compound will exhibit less vascularization than a control animal or an experimental animal receiving a less- or non-effective dose of compound.

[0135] An *in vivo* system designed to test compounds for their ability to limit the spread of primary tumors is described by Crowley *et al.* [Proc. Natl. Acad. Sci. 90:5021-5025, 1993]. Nude mice are injected with tumor cells (PC3) engineered to express CAT (chloramphenicol acetyltransferase). The cells secrete large amounts of uPA and exhibit saturating amounts of uPA activity bound to uPAR on the cell surface. Compounds to be tested for their ability to decrease tumor size and/or metastases are administered to the animals, and subsequent measurements of tumor size and/or metastatic growths are made. In addition, the level of CAT detected in various organs provides an indication of the ability of the test compound to inhibit metastasis; detection of less CAT in tissues of a treated animal versus a control animal indicates less CAT-expressing cells migrated to that tissue.

[0136] *In vivo* experimental models designed to evaluate the urokinase inhibitory potential of a test compound, using a tumor cell line F3II, said to be highly invasive, are described by Alonso *et al.* [Breast Canc. Res. Treat. 40:209-223, 1996]. This group describes *in vivo* studies for toxicity determination, tumor growth, invasiveness, spontaneous metastasis, experimental lung metastasis, and an angiogenesis assay.

[0137] The CAM model (chick embryo chorioallantoic membrane model), first described by L. Ossowski in 1998 [J. Cell Biol. 107:2437-2445, 1988], provides another method for evaluating the urokinase inhibitory activity of a test compound. In the CAM model, invasion of tumor cells through the chorioallantoic membrane is dependent upon the presence of catalytically active uPA. Contacting CAM with tumor cells in the presence of a urokinase inhibitory agent results in less or no invasion of the tumor cells through the membrane. Thus, the CAM assay is performed with CAM and

tumor cells in the presence and absence of various concentrations of test compound. The invasiveness of tumor cells is measured under such conditions to provide an indication of the compound's urokinase inhibitory activity. A compound having urokinase inhibitory activity correlates with less tumor invasion.

[0138] The CAM model is also used in a standard assay of angiogenesis (i.e., effect on formation of new blood vessels (Brooks, P.C.; Montgomery, A.M.P.; and Cheresh, D.A., Methods in Molecular Biology 129: 257-269 (1999)). According to this model, a filter disc containing an angiogenesis inducer, such as basic fibroblast growth factor (bFGF) is placed onto the CAM. Diffusion of the cytokine into the CAM induces local angiogenesis, which may be measured in several ways such as by counting the number of blood vessel branch points within the CAM directly below the filter disc. The ability of compounds of the present invention to inhibit cytokine-induced angiogenesis can be tested using this model. A test compound can either be added to the filter disc that contains the angiogenesis inducer, be placed directly on the membrane or be administered systemically. The extent of new blood vessel formation in the presence and/or absence of test compound can be compared using this model. The formation of fewer new blood vessels in the presence of a test compound would be indicative of anti-angiogenesis activity. Since certain of the compounds of the present invention are active as inhibitors of urokinase, anti-angiogenesis activity for such compounds may suggest that urokinase plays a significant role in angiogenesis.

4. Pharmaceutical Compositions

[0139] In another aspect, the present invention encompasses pharmaceutical compositions prepared for storage or administration which comprise a therapeutically effective amount of a compound of the present invention in a pharmaceutically acceptable carrier.

[0140] The therapeutically effective amount of a compound of the present invention will depend on the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

[0141] The therapeutically effective amount of the compound of the present invention can range broadly depending upon the desired affects and the therapeutic indication. Typically, dosages will be between about 0.01 mg/kg and 100 mg/kg body weight, preferably between about 0.01 and 10 mg/kg, body weight.

[0142] Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid may be added as preservatives. Id. at 1449. In addition, antioxidants and suspending agents may be used. Id.

[0143] The pharmaceutical compositions of the present invention may be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions and suspensions for injectable administration; and the like. The dose and method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

[0144] When administration is to be parenteral, such as intravenous on a daily basis, injectable pharmaceutical compositions can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, or the like. In addition, if desired, the injectable pharmaceutical compositions may contain minor amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents, and the like. If desired, absorption enhancing preparations (e.g., liposomes) may be utilized.

5. Utility

[0145] The compounds of the present invention having urokinase inhibitory activity and/or activity in reducing or inhibiting blood vessel formation, including angiogenesis and neovascularization, may be used both in vitro and *in vivo* for a number of applications, some of which are described herein below.

[0146] The compounds of the present invention are active as inhibitors of urokinase and specifically bind urokinase. Accordingly those compounds that contain sites suitable for linking to a solid/gel support may be used in vitro for affinity chromatography to purify urokinase from a sample or to remove urokinase from a sample using conventional affinity chromatography procedures. These compounds are attached or coupled to an affinity chromatography either directly or through a suitable linker support using conventional methods. See, *e.g.* Current Protocols in Protein Science, John

Wiley & Sons (J.E. Coligan et al., eds, 1997) and Protein Purification Protocols, Humana Press (S. Doonan, ed., 1966) and references therein.

[0147] The compounds of the present invention having urokinase inhibitory activity are useful in in vitro assays to measure tPA activity in a sample. In assays which measure the total plasminogen activation activity in a blood sample, a compound of the present invention having urokinase inhibiting activity will knock out that portion of plasminogen activation attributable to uPA, which will allow for calculation of the portion of the total plasminogen activation due to tPA activity as well as that due to uPA activity. Use of such assays to monitor tPA activity would allow better dosage control in patients receiving tPA. These assays could also be used to monitor uPA activity levels in tissue samples, such as from biopsy or to monitor uPA/tPA activities for any clinical situation where measurement of plasminogen activation activity is of assistance. These assays may also be used to monitor plasminogen activator activity where a patient has been treated with a non-endogenous compound having plasminogen activator activity, such as streptokinase and staphlyokinase.

[0148] The compounds of the present invention are useful *in vivo* for treatment of pathologic conditions which would be ameliorated by decreased urokinase activity. For example these compounds will inhibit the activation of metalloproteases by the uPA-plasmin cascade in synovial fluid and thus, may be used in treatment of arthritis.

[0149] It is believed these compounds will be useful in decreasing or inhibiting metastasis, neovascularization, and degradation of the extracellular matrix in tumors and other neoplasms. These compounds will be useful as therapeutic agents in treating conditions characterized by pathological neovascularation such as retinal disease, retinopathies and other conditions, including those described hereinabove in the Background and Introduction to the Invention.

[0150] Another use for the compounds of the present invention having urokinase inhibitory activity is as an antidote if too much exogenous urokinase has been given to a patient for therewith purposes, such as for dissolving a blood clot.

[0151] The compounds of the present invention may be used in treating conditions characterized by inflammation due to their anti-inflammatory effects from inhibition of urokinase, thereby interfering with mediators of cell adhesion or migration. Such anti-inflammatory applications include treatment of stroke and complications of organ transplants.

[0152] The present invention includes methods for preventing or treating a condition in a mammal suspected of having a condition which will be attenuated by inhibition of urokinase activity comprising administering to said mammal a therapeutically effective amount of a compound or a pharmaceutical composition of the present invention.

[0153] The compounds or pharmaceutical compositions of the present invention are administered *in vivo*, ordinarily in a mammal, preferably in a human. In employing them *in vivo*, the compounds or pharmaceutical compositions can be administered to a mammal in a variety of ways, including orally, parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperitoneally, employing a variety of dosage forms. Administration is preferably oral, such as by tablets capsules or elixirs taken on a daily basis.

[0154] In practicing the methods of the present invention, the compounds or pharmaceutical compositions of the present invention are administered alone or in combination with one another, or in combination with other therapeutic or *in vivo* diagnostic agents.

[0155] As is apparent to one skilled in the medical art, a "therapeutically effective amount" of the compounds or pharmaceutical compositions of the present invention will vary depending upon the age, weight and mammalian species treated, the particular compounds employed, the particular mode of administration and the desired affects and the therapeutic indication. Because these factors and their relationship to determining this amount are well known in the medical arts, the determination of therapeutically effective dosage levels, the amount necessary to achieve the desired result of inhibiting uPA activity, will be within the ambit of one skilled in these arts. Typically, administration of the compounds or pharmaceutical composition of the present invention is commenced at lower dosage levels, with dosage levels being increased until the desired effect of inhibiting uPA activity to the desired extent is achieved, which would define a therapeutically effective amount. For the compounds of the present invention, alone or as part of a pharmaceutical composition, such doses are between about 0.01 mg/kg and 100 mg/kg body weight, preferably between about 0.01 mg/kg and 10 mg/kg, body weight.

[0156] To assist in understanding, the present invention will now be further illustrated by the following examples. These examples as they relate to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

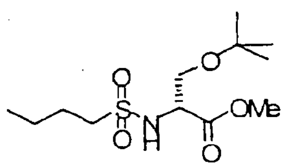
Examples

A. Synthesis of Certain Compounds of the Present Invention

Example 1

Preparation of n-butylsulfonyl-D-serine(tert-butylether)-methyl ester (1)

[0157]

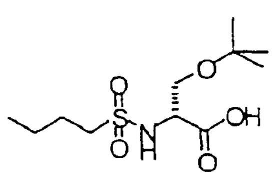


[0158] A solution of HCl·H-dSer(tBu)-OMe (2 g, 9.44 mmol) and n-butylsulfonyl chloride (1.1 ml, 8.50 mmol) in tetrahydrofuran (38 ml) was stirred for ten minutes at room temperature. Diisopropylethylamine (5.75 ml, 33.07 mmol) was then added and the cloudy yellow solution was stirred over night at ambient temperature. The reaction mixture was then diluted with ethylacetate (200 ml) and washed with 1N HCl, followed by brine (20 ml each). After drying over anhydrous sodium sulfate, the solvents were removed under vacuum. The flaky yellow solid (1.56 g, 62%) was judged pure by tlc (5% ethylacetate in hexanes).

Example 2

Preparation of n-butylsulfonyl-D-serine(tert-butylether) (2)

[0159]

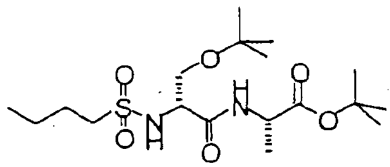


[0160] To a solution of compound 1 (1.46 g, 4.94 mmol) in dioxane (32.95 ml), was added dropwise 2.0 N LiOH (5.44 ml, 10.87 mmol). The cloudy yellow solution was allowed to stir at ambient temperature overnight. When no starting material was observed by tlc (5% ethylacetate/hexanes), the excess dioxane was removed *in vacuo*. The reaction mixture was diluted with a 1:1 mixture of water and methanol and passed through a pre-washed DOWEX (50 × 8-400) ion exchange resin (30 ml). The resin was rinsed thoroughly with methanol and water. The combined filtrates were concentrated under reduced pressure to afford 1.44 g of the title compound in quantitative yield as a cream solid.

Example 3

Preparation of n-butylsulfonyl-D-serine(tert-butylether)-alanine tert-butylester (3)

[0161]

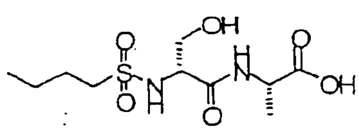


[0162] A solution of the compound of Example 2 (0.50 g, 1.79 mmol), alanine tert-butylester hydrochloride salt (0.65 g, 3.58 mmol), EDC (0.68 g, 3.57 mmol), N-hydroxybenzotriazole (0.27 g, 1.79 mmol) and diisopropylethylamine (1.56 ml, 8.94 mmol) was stirred in acetonitrile (18 ml) at ambient temperature. After 18 hours, the solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate (50 ml) and 1N HCl (10 ml). The ethylacetate layer was washed with 1N HCl (10 ml), saturated sodium bicarbonate (2 × 15 ml) and brine (15 ml), then dried with sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography eluting with 50% ethyl acetate/ hexanes, yielding 429 mg (59%) product. The product was a single peak by reverse phase (C18) HPLC (t_R= 9 minutes at 0.1% trifluoroacetic acid in 5-90% aqueous acetonitrile over 20 minutes).

Example 4

Preparation of n-butylsulfonyl-D-serine-alanine (4)

[0163]

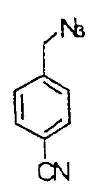


[0164] To a solution of the compound of Example 3 (0.42 g, 1.02 mmol) in dichloromethane (4.2 ml) was added trifluoroacetic acid (4.2 ml). The reaction mixture was stirred at ambient temperature for 1 hour. The reaction mixture was diluted with 50 ml n-heptane and concentrated *in vacuo.* The residue was resuspended in 10 ml acetonitrile and 50 ml n-heptane and concentrated *in vacuo* to yield 410 mg product.

Example 5

Preparation of α-azido-4-cyanotoluene (5)

[0165]

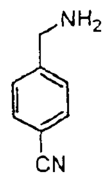


[0166] Sodium azide (Aldrich, 3.5 g, 54 mmol) was added to a solution of α-Bromotoluenitrile (Aldrich, 10 g, 51 mmol) in DMF (100 ml), and the resulting mixture was stirred at ambient temperature for 5 hours. The reaction mixture was then diluted with water (350 ml) and extracted with ether (2 x 100 ml). The combined organic layers were washed with brine and dried ($MgSO_4$). Removal of solvent led to the title compound (8 g, 96%). ^1H NMR ($CDCl_3$) : δ 4.42 (s, 2H), 7.41 (d, 2H, J = 8.1 Hz), 7.65 (d, 2H, J = 8.1 Hz).

Example 6

Preparation of 4-(aminomethyl)benzylnitrile (6)

[0167]

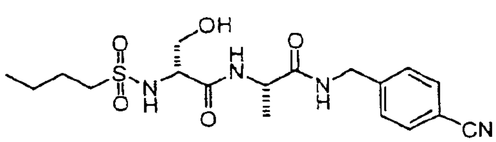


[0168] The catalyst of 10% Pd-on-C (Aldrich, 800 mg) was added to a solution of α-azido-4-cyanotoluene (compound **5**, 8 g, 51 mmol) in EtOAc (150 ml). The reaction mixture was hydrogenated (H_2, 45 psi) in a Parr apparatus for 11 hours. Catalyst was filtered and the solvent was removed under vacuum to give the title compound (6.3 g, 93%). ^1H NMR ($CDCl_3$) : δ 3.85 (s, 2H), 7.45 (d, 2H, J = 8.1), 7.60 (d, 2H, J = 8.1 Hz), 7.78 (s, 2H, NH_2).

Example 7

Preparation of n-butylsulfonyl-D-serine-alanine-4-cyanobenzylamide (7)

[0169]

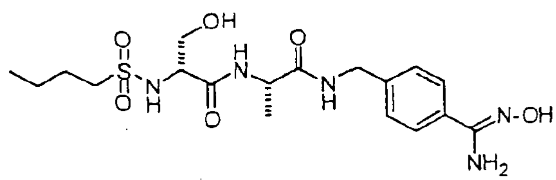


[0170] A solution of compound **4** (150 mg, 0.51 mmol), 4-(aminomethyl)benzylnitrile (compound **6**, 171 mg, 1.02 mmol), EDC (195 mg, 1.02 mmol), and N-hydroxybenzotriazole (78 mg, 0.51 mmol) in acetonitrile (5.1 ml) was stirred at ambient temperature for 10 minutes. 2,4,6-Collidine (0.34 ml, 2.54 mmol) was then added and the reaction mixture was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate (100 ml) and 0.5M HCl (10 ml). The ethylacetate layer was washed with water followed by 0.5M HCl (10 ml), saturated sodium bicarbonate (2 × 10 ml) and brine (15 ml), then dried with sodium sulfate. The solvent was removed under reduced pressure to yield 237 mg product. The product eluted at 9.5 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes.

Example 8

Preparation of n-butylsulfonyl-D-serine-alanine-4-hydroxyamidinobenzylamide **(8)**

[0171]

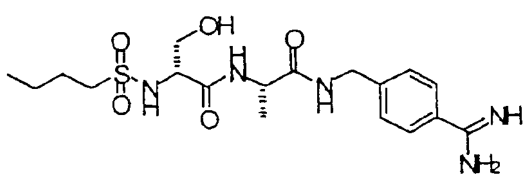


[0172] To a solution of the product in Example 7 (117 mg, 0.285 mmol) in 1.14 ml methanol was added hydroxylamine hydrochloride (33.7 mg, 0.485 mmol), followed by N-methylmorpholine (53 μl, 0.485 mmol). The reaction mixture was stirred overnight at ambient temperature and then at 50°C for six hours. The reaction mixture was concentrated *in vacuo*. The crude product was taken to the next step (Example 9) without further purification. The product eluted at 6.5 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-50% aqueous acetonitrile over 20 minutes.

Example 9

Preparation of n-butylsulfonyl-D-serine-alanine-4-amidinobenzylamide (9)

[0173]

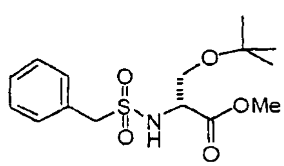


[0174] To the product of Example 8 (126 mg, 0.285 mmol) in acetic acid (2.85 ml) and water (0.28 ml) was added 185 mg activated zinc dust. The reaction mixture was stirred overnight at room temperature. The zinc dust was filtered using a glass funnel and the filtrate was purified by preparative HPLC. The fractions containing the product eluted in a 5-20% aqueous acetonitrile containing 0.1% TFA and were pooled and lyophilized yielding 35 mg of the title compound as a white powder. The product eluted at 6.0 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-50% aqueous acetonitrile over 20 minutes.

Example 10

Preparation of benzylsulfonyl-D-serine(tert-butylether)-methyl ester **(10)**

[0175]

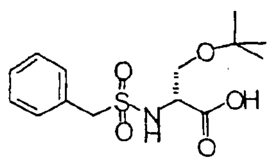


[0176] A solution of HCl·H-dSer(tBu)-OMe (1 g, 4.72 mmol) and phenethylsulfonyl chloride (1.45 g, 7.08 mmol) in acetonitrile (19 ml) was stirred for ten minutes at room temperature. Diisopropylethylamine (1.53 ml, 11.81 mmol) was then added and the clear yellow solution was stirred for 18 hours at ambient temperature. The reaction mixture was then diluted with ethylacetate (100 ml) and washed with 1N HCl, followed by brine (10 ml each). After drying over anhydrous sodium sulfate, the solvents were removed *in vacuo*. The crude product was purified by flash column chromatography eluting with dichloromethane, followed by a gradient consisting of 1 to 5% ethylacetate in dichloromethane, yielding 840 mg (52%) product. Tlc of the final product in 5% ethylacetate in dichloromethane gave one spot with an Rf of 0.52.

Example 11

Preparation of benzylsulfonyl-D-serine(tert-butylether) **(11)**

[0177]

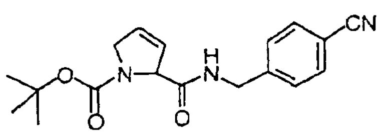


[0178] To a solution of the compound of Example 10 (1.0 g, 3.03 mmol) in dioxane (20 ml), was added dropwise 2.0N LiOH (3.33 ml, 6.67 mmol). The solution was allowed to stir at ambient temperature overnight. The excess dioxane was removed *in vacuo*. The reaction mixture was diluted with a 1:1 mixture of water and methanol and passed through a pre-washed DOWEX (50 x 8-400) ion exchange resin (30 ml). The resin was rinsed thoroughly with methanol and water. The combined filtrates were concentrated under reduced pressure to afford 1.10 g of the title compound as a yellow glue.

Example 12

Preparation of tert-butyloxycarbonyl-3,4-dehydroproline p-cyanobenzylamide **(12)**

[0179]

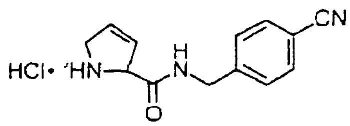


[0180] A solution of tert-butyloxycarbonyl-3,4-dehydroproline (0.4 g, 1.88 mmol), 4-(aminomethyl)benzylnitrile (compound 6, 0.47 g, 2.82 mmol), EDC (0.54 g, 2.82 mmol), N-hydroxybenzotriazole (0.29 g, 1.88 mmol), and diisopropylethylamine (1.64 ml, 9.39 mmol) in acetonitrile (7.5 ml) was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate (25 ml) and 0.5M HCl (5 ml). The ethylacetate layer was washed with water followed by 0.5M HCl (5 ml), saturated sodium bicarbonate (2 × 5 ml) and brine (10 ml), then dried with sodium sulfate. The solvent was removed under reduced pressure and the crude was purified by flash column chromatography eluting with 4/1 ethylacetate/hexane to yield 561 mg pure product (91.3%). The product eluted at 10.5 minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-75% aqueous acetonitrile over 20 minutes.

Example 13

Preparation of 3,4-dehydroproline-4-cyano benzylamide hydrochloride salt (13)

[0181]

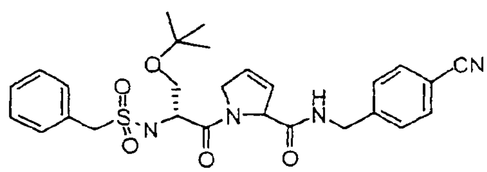


[0182] To a solution of the compound of Example 12 (0.47 g, 1.44 mmol) in ethylacetate (5.7 ml) was added 5M anhydrous HCl in ethylacetate (1.44 ml) and the reaction was stirred at ambient temperature overnight. The reaction mixture was concentrated *in vacuo* to yield 363 mg (95%) of a white solid.

Example 14

Preparation of benzylsulfonyl-D-serine(tert-butylether)-proline(dehydro)-4-cyanobenzylamide (14)

[0183]

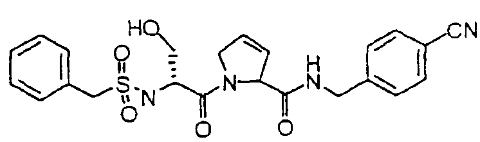


[0184] A solution of the compound of Example 11 (0.10 g, 0.32 mmol), the compound of example 13 (0.092 g, 0.34 mmol), EDC (0.091 g, 0.48 mmol), and N-hydroxybenzotriazole (0.053 g, 0.35 mmol) was stirred in acetonitrile (1.2 ml) for 10 minutes. 2,4,6-Collidine (0.209 ml, 1.58 mmol) was then added and the reaction mixture was stirred over night at ambient temperature. The solvent was removed under reduced pressure. The resulting residue was resuspended in ethylacetate (50 ml) and 1N HCl (10 ml). The ethylacetate layer was washed with 1N HCl (10 ml), saturated sodium bicarbonate (2 × 15 ml) and brine (15 ml), then dried with sodium sulfate to a yellow syrup (160 mg, 94%). The product was a single peak by reverse phase (C18) HPLC (t_R= 11 minutes at 0.1% trifluoroacetic acid in 5-90% aqueous acetonitrile over 20 minutes).

Example 15

Preparation of benzylsulfonyl-D-serine-3,4-dehydroproline-4-cyanobenzylamide (15)

[0185]



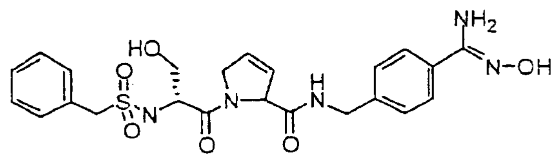
[0186] To a solution of the compound of Example 14 (0.16 g, 0.30 mmol) in dichloromethane (0.6 ml) was added trifluoroacetic acid (0.6 ml). The reaction mixture was stirred at ambient temperature for 1 hour. The reaction mixture was diluted with 10 ml n-heptane and concentrated *in vacuo*. The residue was resuspended in 5 ml acetonitrile and

10 ml n-heptane and concentrated *in vacuo* to yield 183 mg product.

Example 16

Preparation of benzylsulfonyl-D-serine-3,4-dehydroproline-4-hydroxyamidinobenzylamide **(16)**

[0187]



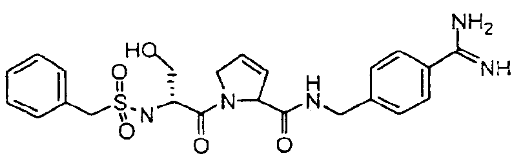
[0188] To a solution of the product in Example 15 (143 mg, 0.305 mmol) in 1.22 ml methanol was added hydroxylamine hydrochloride (0.036 mg, 0.519 mmol) followed by N-methylmorpholine (57 µl, 0.519 mmol). The reaction mixture was stirred over night at ambient temperature. Analytical HPLC suggested that the reaction was not complete. Additional hydroxylamine hydrochloride (0.036 mg, 0.519 mmol) and N-methylmorpholine (57

[0189] 1, 0.519 mmol) were added and stirring continued at ambient temperature over night. The reaction mixture was concentrated *in vacuo* and the crude was purified by preparative HPLC. The fractions containing the product eluting in 5-20% aqueous acetonitrile containing 0.1% TFA solution were pooled and lyophilized yielding 16 mg of the title compound as a white powder.

Example 17

Preparation of benzylsulfonyl-D-serine-3,4-dehydroproline-p-amidinobenzylamide **(17)**

[0190]

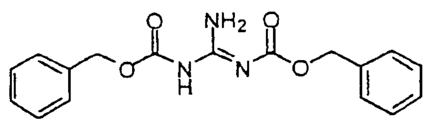


[0191] To the product of Example 16 (15 mg, 0.030 mmol) in acetic acid (0.30 ml) and water (0.03 ml) was added 19 mg activated zinc dust. The reaction mixture was stirred over night at room temperature. The zinc dust was filtered using a glass funnel and the filtrate was purified by preparative HPLC. The fractions containing the product eluted in a 5-20% aqueous acetonitrile containing 0.1% TFA, and were pooled and lyophilized yielding 7 mg of the title compound as a white powder. The product eluted at minutes by reverse phase (C18) HPLC at 0.1% trifluoroacetic acid in 5-50% aqueous acetonitrile over 20 minutes.

Example 18

Preparation of bis(benzyloxycarbonyl)guanidine (18)

[0192]



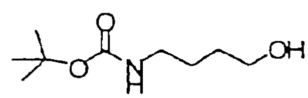
[0193] The synthesis of the title product was carried out as cited in the literature (tetrahedron Letters, vol. 35, No. 7, pp. 977-980, 1994) and is described below:

[0194] A solution of N,N'-bis(benzyloxycarbonyl)-S-methylisothiourea (1 g, 2.79 mmol) in 7N anhydrous ammonia in methanol (5.5 ml) was stirred overnight at ambient temperature. The reaction mixture was concentrated and the remaining residue was diluted with ethylacetate (10 ml). The organic layer was washed twice with saturated sodium bicarbonate and once with brine (10 ml each). After drying over sodium sulfate, the crude product was subjected to flash column chromatography eluting with 3/2 ethylacetate/hexanes to yield 0.87 g of a white solid. The product was then recrystallized in 1:1 ethylacetate: hexane solvent system to yield 337 mg (37%) pure product (mp= 151 °C).

Example 19

Preparation of tert-butyloxycarbonyl-4-amino-1-butanol (19)

[0195]

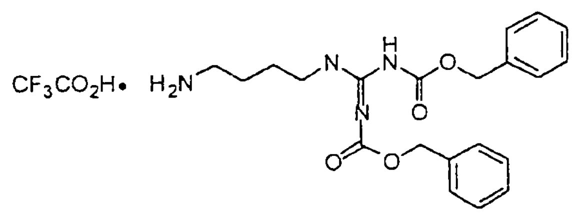


[0196] To a solution of 4-amino-1-butanol (1 g, 11.22 mmol) in tetrahydrofuran (45 ml) was added Boc-anhydride (2.20 g, 10.10 mmol) and triethylamine (2.84 g, 28.04 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and the remaining residue was diluted with ethylacetate (250 ml) and 1M HCl (50 ml). The layers were separated and the organic layer was washed with 1N HCl, water and brine (50 ml each). After drying over sodium sulfate 1.9 g (99%) product was obtained as a clear oil.

Example 20

Synthesis of g-N,N'-bis(benzyloxycarbonyl)agmatine trifluoroacetate salt (20)

[0197]

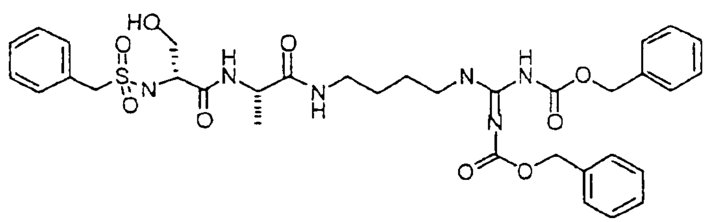


[0198] To a solution of compound **18** (the product of Example 18) (0.2 g, 0.61 mmol) and triphenylphosphine (0.12 g, 0.46 mmol) in dry toluene (6.6 ml) under nitrogen was added via syringe compound **19** (the product of Example 19). The mixture was cooled to 0°C, and diethylazodicarboxylate (0.080 g, 0.46 mmol) was added dropwise over 15 minutes. The reaction mixture was stirred at room temperature over night at which time tlc in 3/2 ethylacetate/hexane confirmed the completion of the reaction. Five drops of water were added and the solvent was evaporated *in vacuo*. The crude was purified by flash column chromatography eluting with 9/1 hexanes/ethylacetate, followed by 3/2 hexanes/ethylacetate to yield 83 mg (74%) pure product. The product was then treated with dichloromethane and trifluoroacetic acid (1 ml each) for one hour at ambient temperature. Removal of the solvents *in vacuo* afforded 80 mg product **20**.

Example 21

Preparation of n-butylsulfonyl-D-serine-alanine-aqmatine-g-N,N-bis(benzyloxycarbonyl) **(21)**

[0199]

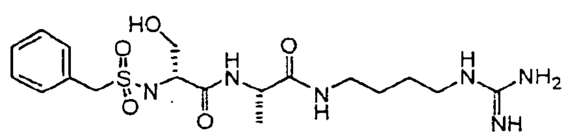


[0200] A solution of the compound of Example 4 (0.05 g, 0.17 mmol), and the compound of Example 20 (0.065 g, 0.17 mmol), EDC (0.065 g, 0.34 mmol), and hydroxybenzotriazole (0.026 g, 0.17 mmol) was stirred in acetonitrile (0.67 ml) for 10 minutes. 2,4,6-Collidine (0.11 ml, 0.84 mmol) was then added and the reaction mixture was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate and 1N HCl (5 ml each). The ethylacetate layer was washed with 1N HCl (5 ml), saturated sodium bicarbonate (2 × 5 ml) and brine (5 ml), then dried with sodium sulfate to a solid (114 mg, 94%).

Example 22

Preparation of n-butylsulfonyl-D-serine-alanine-agmatine **(22)**

[0201]

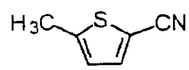


[0202] The compound of Example 21 (114 mg, 0.17 mmol) was dissolved in methanol (15 ml) and was hydrogenated on a Parr shaker overnight at 40 psi in the presence of 15 mg palladium on charcoal. The catalyst was filtered off. The reaction mixture was diluted to 35 ml with water and purified by preparative HPLC. The fractions containing the product eluted in 0-25% aqueous acetonitrile containing 0.1% TFA and were pooled and lyophilized yielding 16 mg of the title compound as a white powder.

Example 23

Preparation of 2-cyano-5-methylthiophene **(23)**

[0203]

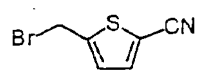


[0204] A solution of 2-bromo-5-methylthiophene (TCI chemicals, 5 g, 28 mmol) and copper(I) cyanide (Aldrich, 2.53 g, 28 mmol) in DMF (10 ml) was heated at its reflux temperature for 4 hours. After cooling to ambient temperature, ethyl acetate (500 ml) and 10% NaCN aqueous solution (500 ml) was added. After separation, the aqueous layer was extracted with ethyl acetate (300 ml), and the combined organic layers were concentrated to an oil. The oil was further purified by a flash column chromatography (ethyl acetate) to give the title compound (3.03 g, 87%). TLC: Rf 0.30 (1:1 of hexane/ethyl acetate); ^{1}H NMR ($CDCl_3$) : δ 2.55 (m, 3H), 6.76 (d, 1H, J = 3.6 Hz), 7.42 (d, 1H, J = 3.6 Hz).

Example 24

Preparation of 2-cyano-5-(bromomethyl)thiophene **(24)**

[0205]

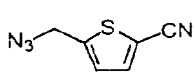


[0206] A solution of 2-cyano-5-methylthiophene (compound **23**, 3.0 g, 24 mmol), N-bromosuccinimide (Aldrich, 4.8 g, 27 mmol), and 2,2'-azobisisobutyronitrile (Aldrich, 0.4 g, 2.4 mmol) in CCl_4 (Aldrich, 60 ml) was heated at its reflux temperature for 5 hours. After cooling to ambient temperature, the solvent was removed under vacuum to give a yellow oil. The oil was purified by a flash column chromatography (1:1 of hexane/ethyl acetate) to give the title compound (4.5 g, 91%). TLC: Rf 0.91 (1:1 of hexane/ethyl acetate); ^{1}H NMR ($CDCl_3$): δ 4.66 (s, 2H), 7.10 (d, 1H, J = 3.8 Hz), 7.48 (d, 1H, J = 3.8 Hz).

Example 25

Preparation of 2-cyano-5-(azidomethyl)thiophene **(25)**

[0207]

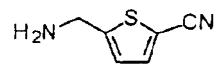


[0208] A solution of 2-cyano-5-(bromomethyl)thiophene (compound **24**, 3.5 g, 17.3 mmol) and sodium azide (Aldrich, 1.7 g, 26 mmol) in DMF (Aldrich, 60 ml) was stirred at ambient temperature for 10 hours. Flash column chromatography (20% ethyl acetate in hexane) resulted the title compound (2.35 g, 83%). TLC: Rf 0.48 (20% of ethyl acetate in hexane) ; ^{1}H NMR ($CDCl_3$) : δ 4.56 (s, 2H), 7.01 (d, 1H, J = 3.7 Hz), 7.55 (d, 1H, J = 3.7 Hz).

Example 26

Preparation of 2-cyano-5-(aminomethyl)thiophene (26)

[0209]

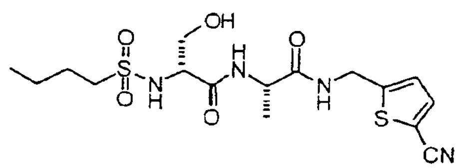


[0210] Triphenylphosphine (Aldrich, 5.7 g) was added to a solution of 2-cyano-5-(azidomethyl)thiophene (compound **25**, 2.5 g, 10 mmol) in THF (Aldrich, 40 ml) and water (10 ml) at 0°C. The resulting solution was allowed to warm to room temperature and stirred at ambient temperature for 10 hours. RP HPLC purification led to the title compound (2.3 g, 94%). MS (electrospray): 139 (M + 1); ^{1}HNMR ($CDCl_3$) : δ 4.01 (s, 2H), 4.75 (br s, 2H, NH_2), 6.82 (d, 1H, J = 3.5 Hz), 7.08 (d, 1H, J = 3.5 Hz).

Example 27

Preparation of n-butylsulfonyl-D-serine-alanine-2-cyano-5-(methylamide)thiophene (27)

[0211]



[0212] A solution of the compound of example 4 (**4**, 860 mg, 2.9 mmol), and the compound of Example **26** (26, 400 mg, 2.9 mmol), EDC (556 mg, 2.9 mmol), N-hydroxybenzotriazole (488 mg, 3.19 mmol) and diisopropylethylamine (1.5 ml, 8.7 mmol) was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the resulting residue was resuspended in ethylacetate (50 ml) and 1N sodium bisulfate (10 ml). The ethylacetate layer was washed with 1N sodium bisulfate (10 ml), saturated sodium bicarbonate (2 x 15 ml) and brine (15 ml), then dried with sodium sulfate to give a yellow oil. The crude product was subjected to flash column chromatography eluting with

@PJL SET FINISH=NONE
@PJL SET PAGEPROTECT=AUTO
@PJL SET PAPER=LETTER
@PJL SET HOLD=OFF
@PJL SET RESOLUTION=300
@PJL SET EDGETOEDGE=NO
@PJL ENTER LANGUAGE=PCL